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Susceptibilities of *Mycoplasma gallisepticum* and *Mycoplasma synoviea* Isolates to Antimicrobial Agents *in vitro*

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Abstract: The *in vitro* activities of aivlosin tylosin tiamulin, erythromycin, oxytetracycline, spectinomycin, lincomycin, lincomycin-spectinomycin (1:2) and enrofloxacin were determined against twenty-eight isolates of *Mycoplasma gallisepticum* and 4 isolates of *Mycoplasma synoviae* using microbroth dilution method. The isolates showed various susceptibilities to antimicrobial agents. Aivlosin, lincomycin-spectinomycin (1:2), tylosin, tiamulin, enrofloxacin and also lincomycin were commonly more effective against these field isolates. However, aivlosin was the most effective drugs against *Mycoplasma gallisepticum* and *Mycoplasma synoviae* as its MIC was the lowest of all. Oxytetracycline, erythromycin and spectinomycin were not effective against all isolates, as their MICs for some of isolates were extremely high. The MIC of erythromycin and oxytetracycline were distributed across a broad range. Resistant strains to two mentioned antibiotics were obtained from the field.

Key words: *Mycoplasma* sp., antimicrobial agents, poultry

INTRODUCTION

Mycoplasma gallisepticum and *Mycoplasma synoviae* has long been recognized as common respiratory pathogens especially in chickens causing lots of commercial losses in poultry industries. *Mycoplasma gallisepticum* infection commonly induces chronic respiratory disease in chickens (Ley, 2003).

The clinical signs include nasal discharge, sneezing, coughing, tracheal rales and mild conjunctivitis. *Mycoplasma synoviae* most frequently occurs as a subclinical upper respiratory infection but may result in airsacculitis and synovitis in chickens and turkeys (Kleven, 2003).

Establishing of the *Mycoplasma gallisepticum* and *Mycoplasma synoviae*-clean status of breeder flocks and maintaining that status of can be accomplished by participation in control programs (Kleven, 2003; Ley, 2003). Like other countries in Iran chicken primary and multiplier breeders and hatcheries generally have adopted various mycoplasma control programs. In spite of these preventive programs, a lot of broiler and layer flocks will be affected by those organisms during their production periods. However , line and parent flocks are mycoplasma free, but a large number of breeder flocks also involve *Mycoplasma gallisepticum* and or *Mycoplasma synoviae* infections. However, it is not widely allowed the use of lived mycoplasma vaccines in the country, control of mycoplasma infections by vaccination is limited.

Control of these infections by chemotherapy is sometimes necessary in complement of biosecurity measures to minimize economic losses and lateral and vertical transmissions. Here the most of veterinarians prescript the consumption of antibiotics on the basis of clinical findings and their experiences in order to treatment affected flocks and improve egg production rate. It is logical that for a successful and aimed mycoplasma infection treatment, it is necessary to have regular antibiogram tests of *Mycoplasma gallisepticum* and or *Mycoplasma synoviae* in the field for monitoring susceptibility of Mycoplasma prevalent in the farms.

Antibiotics: Basically *Mycoplasma gallisepticum* and *Mycoplasma synoviae* have shown sensitivity *in vitro* and *in vivo* to several antimicrobials including macrolides (Jordan and Horrocks, 1996) tetracyclines and quinolones (Bébéar *et al*., 1999; Wu *et al*., 2000), but they are resistant to penicillins or other antibiotic inhibitors of cell wall synthesis.

On the other hand many antimicrobial agents, such as oxytetracycline, amino glycosides lincosamides, fluoroquinolones and tiamulin have been shown to possess different degrees of *in vitro* activity against various veterinary mycoplasmas (Bradbury *et al*., 1994; Hannan *et al*., 1997a). However, increasing resistance of mycoplasmas against tetracyclines (Hannan *et al*. 1997a) macrolides (Bradbury *et al*., 1994; Hannan *et al*., 1997a; Gautier-Bouchardon *et al*., 2002) and quinolones (Bébéar *et al*., 1999; Wu *et al*., 2000) has been reported in animal and human species.

MATERIALS AND METHODS

On this way we carried out a 2 stage research, at first stage isolation and molecular identification of

Mycoplasma gallisepticum and *Mycoplasma synoviae* were done. Isolation was carried out by enriched mycoplasma broth and agar. One hundred mycoplasma isolates were identified genetically by PCR and RFLP (Garcia *et al*., 1995; Ghaleh *et al*., 2005). All of isolates gathered in Fars province of Iran and related tests carried out in Shiraz Veterinary Medicin School, Shiraz, Iran.

Test organisms: In the second stage the antibiogram test were carried out on 28 field isolates of *Mycoplasma gallisepticum* and 4 field isolates *Mycoplasma synoviae* by common antibiotics. The antibiogram test was designed on the basis of micro dilution method in broth medium (Whithear *et al*., 1983; Hannan *et al*., 1997b). Although, considerable data has been obtained on the *in vitro* antibiotic susceptibility of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* and other avian mycoplasmas abroad but no information is available about the susceptibility of these organisms in Iran. All isolates were between the 20th and 30th medium passage.

Antibiotics: Antibiotics used to determine the sensitivity of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* isolates, were Aivlosin tartrate (E.Co.A.H.) Tylosin tartrate (Sigma), Tiamulin base (ACS Chemicals), Erythromycin base (Merck), Oxytetracycline dihydrate (Sigma), Spectinomycin dihydrochloride (Sigma), Lincomycin hydrochloride (Sigma), Lincomycin-Spectinomycin (1:2), Enrofloxacin base (ACS Chemicals). Erythromycin base were dissolved in 7% ethanol. Each antibiotic solution was passed through a 450 nm pore size membrane filter (Millipore, USA) and then dispensed aseptically. FOR every antibiogram test the solutions prepared under sterile conditions and in WFI water as stock solutions. The stock solutions were diluted in mycoplasma broth to dilution two times that required and were dispensed in 1 mL aliquots and then frozen at -20°C until used.

Media: The media used were the mycoplasma broth and or mycoplasma agar. Mycoplasma broth medium was formulated and described by Frey *et al*. (1968). Added glucose (dextrose) to broth medium mycoplasma fermentative growth was enhanced and provided an indicator of growth when glucose fermentation produced acids in the media containing phenol red. The color of medium gradually changed from red to orange or yellow if the growing fermentative species of mycoplasmas existed (Kleven, 2003).

Sometimes the effectiveness of various antibiotics towards *Mycoplasma gallisepticum* and *Mycoplasma synoviea* in the field is not very different but some antibiotics show better advantages. Enrofloxacin still retains the acceptable advantage because it has broader spectrum of activity than the macrolides, being active also against a lot of gram-negative bacteria, which very often complicate *Mycoplasma gallisepticum* and *Mycoplasma synoviea* infections (Ghaleh *et al*., 2005).

At first 0.2 mL was chosen as final volume for each well containing 0.05 mL antibiotic solution, 0.05 mL fresh broth media solution (and then serially diluted) finally 0.1 mL fresh broth culture of each isolates inoculated to each well (which they made 0.2 mL of test volume). However fresh broth culture of each isolates used as primary inoculums, the number of viable mycoplasmas inoculated in each test of sensitivity to the antibiotics was determined by micro-broth dilution using mycoplasma enrichment broth with phenol-red indicator. A titre of 10^3/0.1-10^4 /0.1 mL fresh viable mycoplasma was needed for the test proper which is added to each well without any serial dilution. The change of color in the broth medium from red to yellow following inoculation of isolates and incubation at 37°C for 14 days showed growth of the isolates. A micro-broth dilution procedure which is described by Whithear *et al*. (1983) used in this research except we count the colonies of 24 fresh broth cultures of isolates before starting the sensitivity tests. The test was carried out in 96-well, U bottom micro titration disposable plates with cover under sterile conditions using multichannel micropipette (Ependorffe Co., Germany). At least six and sometimes 10-14 replicate doubling dilutions were made in experiments to evaluate test variables. According to Table 1, 150 µL of mycoplasma enrichment broth (pH 7.4) were added to each well containing final dilution of antibiotic, containing desired density of isolates was inoculated in to each well. Positive and negative culture controls containing mycoplasma enrichment broth plus different isolates without antibiotics and mycoplasma broth plus different isolates and antibiotics and zero culture controls included only mycoplasma enrichment broth (without any isolates and antibiotics), were included in all test plates. Plates were covered with their covers to prevent the evaporation of the broth medium during incubation period at 37°C. As it mentioned above the standardized test microbroth dilution procedure which is described by Whithear *et al*. (1983) was applied for *Mycoplasma gallisepticum* and *Mycoplasma synoviae* isolates. The standardized test was used to determine the sensitivity of 28 strains of *Mycoplasma gallisepticum*, four strains of *Mycoplasma synoviae* to the antibiotics. Mycoplasma broth prepared by the described formula. Erythromycin base were dissolved in 7% ethanol. Each antibiotic solution was passed through a 450 nm membrane filter (Millipore, USA) and then dispensed aseptically. For every antibiogram test antibiotic solutions were prepared freshly and immediately used. Solutions were diluted in mycoplasma broth in two fold dilution started in 100 µg/mL for first time but for second and third time the

Table 1: *In vitro* activities of, oxytetracycline, aivlosin, tylosin, tiamulin, erythromycin, spectinomycin, lincomycin, L/S and enrofloxacin against *Mycoplasma gallisepticum* isolates

Isolate No.	Inoculum No. of Myco /0.2mL	Oxytetra-cycline	Aivlosin	Tylosin	Tiamulin	Erythro-mycin	Spectino-mycin	Linco mycin	L/S	Enrofloxacin
1	$3.0x10^5$	1.56	0.024	0.195	0.78	0.39	0.78	0.78	0.0485/0.097	0.195
2	$2.7x10^5$	1.56	0.024	0.195	0.39	0.195	0.78	0.78	0.0485/0.097	0.390
3	$2.2x10^5$	3.12	0.024	0.195	0.39	0.195	0.78	1.56	0.097/0195	0.195
4	$2.6x10^5$	3.12	0.024	0.195	0.39	0.390	0.78	1.56	0.097/0.195	0.195
5	$4.0x10^5$	3.12	0.024	0.390	0.78	0.390	1.56	1.56	0.097/0.195	0.390
6	$3.1x10^5$	1.56	0.024	0.390	0.78	0.195	1.56	1.56	0.097/0.0194	0.390
7	$2.2x10^5$	3.12	0.024	0.390	0.78	0.195	0.78	0.78	0.0485/0.097	0.390
8	$2.6x10^5$	3.12	0.048	0.390	0.39	0.195	0.78	0.78	0.0485/0.097	0.195
9	$3.3x10^5$	25.00	0.024	0.195	0.39	0.390	1.56	0.78	0.0485/0.097	0.195
10	$1.9x10^5$	12.50	0.024	0.195	0.39	0.390	1.78	0.78	0.0485/0.097	0.195
11	$2.8x10^5$	12.50	0.024	0.390	0.78	0.390	0.78	0.78	0.0485/0.097	0.390
12	$2.0z10^5$	25.00	0.024	0.195	0.39	0.390	0.78	0.78	0.0485/0.097	0.390
13	$3.1x10^5$	6.25	0.048	0.390	0.39	0.390	0.78	0.78	0.0485/0.097	0.390
14	$2.0x10^5$	3.12	0.024	0.195	0.39	0.390	0.78	0.78	0.0485/0.097	0.195
15	$1.5x10^5$	1.56	0.024	0.195	0.39	0.390	0.78	0.78	0.0485/0.097	0.195
16	$1.0 x10^5$	3.12	0.012	0.097	0.78	0.390	1.56	1.56	0.097/0.195	0.195
17	$2.3x10^5$	25.00	0.024	0.195	0.39	0.390	1.56	0.78	0.0485/0.097	0.390
18	$3.6x10^5$	3.12	0.012	0.097	0.39	0.390	0.78	0.78	0.0485/0.097	0.195
19	$3.2x10^5$	3.12	0.024	0.195	0.78	12.500	0.78	0.78	0.0485/0.97	0.390
20	$1.9x10^5$	6.25	0.012	0.097	0.39	0.390	0.78	0.79	0.0485/0.097	0.195
21	$1.7x10^5$	3.12	0.024	0.195	0.39	6.250	1.56	1.56	0.097/0.195	0.195
22	$1.6x10^5$	6.25	0.024	0.195	0.39	0.390	1.56	1.56	0.097/0.195	0.195
25*	$1.6x10^5$	12.50	0.024	0.195	0.78	1.560	0.78	1.56	0.097/0.195	0.390
26	$1.8x10^5$	12.50	0.024	0.195	0.78	0.780	0.78	1.56	0.097/0.195	0.390
27	$3.1x10^5$	25.00	0.024	0.195	0.39	0.390	12.50	0.78	0.0485/0.097	0.195
28	$2.7x10^5$	25.00	0.024	0.195	0.78	6.250	12.50	0.78	0.0485/0.097	0.195
29	$2.2x10^5$	6.25	0.024	0.195	0.39	0.780	6.25	1.56	0.097/0.195	0.195
30*	$2.5x10^5$	6.25	0.024	0.195	0.39	0.780	1.56	1.56	0.097/0.195	0.195

*Isolate numbers 23, 24, 31 and 32 are *Mycoplasma synoviae* (MS) see Table 2

narrower rage of titration was required started in 25, 6.25 and 0.195 µg/mL. The ranges of concentrations of active compounds used in the final tests to survey the sensitivity of avian mycoplasmas to the antibiotics were as follows: Tiamulin 25 µg/mL to 1.56 µg/mL, spectinomycin, oxytetracycline, erythromycin, lincomycin, tylosin and enrofloxacin from 6.25 µg/mL to 0.195 µg/mL to 0.012 µg/mL (Hannan *et al*., 1997a,b). All antibiotic solution pH were adjusted to the pH of the mycoplsma medium.

Antibiogram tests were carried out in 96 well sterilized micro plate U bottoms. When growth occurred in the well, a definite change was observed of the phenol red to yellow (usually after 140 h of incubation) and the minimum inhibitory concentration (MIC) was determined. The MIC was the lowest concentration of antibiotic inhibiting growth (no color change of phenol red).

When the MIC was determined, approximately 0.01 mL of broth from each tube was placed on an agar plate. The plates were incubated at 37°C in a moist container and examined microscopically after 5 days for mycoplasma colonies (Gautier-Bouchardon *et al*., 2002, Bradbury *et al*., 1994). All MIC values were expressed in µg/mL of each active compound. Also all sensitivity tests repeated second times to determine the accuracy of each test.

RESULTS

The *in vitro* activities of lincomycin-spectinomycin combination (1:2), erythromycin, oxytetracycline, lincomycin, spectinomycin, enrofloxacin, tylosin, aivlosin and tiamulin against the 28 isolates of *Mycoplasma gallisepticum* and 4 isolates of *Mycoplasma synoviae*, as determined by the micro-broth technique, are shown in Table 1. Of the eight antimicrobials, oxytetracycline had the highest and aivlosin had the lowest MIC values. The results for *Mycoplasma synoviae* are given in Table 2 and again demonstrate the greater sensitivity of the isolates to aivlosin and lincomycin-spectinomycin than to oxytetracycline. Of the other antimicrobials enrofloxacin, tylosin erythromycin, tiamulin, spectino-mycin and lincomycin showed the lowest MICs with *Mycoplasma synoviae*.

Against 4 isolates of MS by the micro-broth method aivlosin showed the best *in vitro* activity giving MICs of 0.042 µg/mL for most isolates. Also, lincomycin-spectinomycin (1:2) combination had MICs of 0.0485/0.097 µg/mL for most isolates. Tiamulin and erythromycin had similar MICs of 0.39-0.78 µg/mL for the isolates. Spectinomycin and lincomycin had MICs ranging from 0.78-1.56 µg/mL and oxytetracycline had MICs of 6.25-.56 µg/mL.

The combination of lincomycin-spectinomycin (1:2) appeared to have a synergistic effect for all isolates.

Table 2: *In vitro* activities of oxytetracycline, aivlosin, tylosin, tiamulin, erythromycin, spectinomycin, lincomycin, lincomycin-spectinomycin and enrofloxacin against *Mycoplasma synoviae* isolates

Isolate No.	Inoculum No. of Myco /0.2mL	Oxytetra-cycline	Aivlosin	Tylosin	Tiamulin	Erythro-mycin	Spectino-mycin	Linco mycin	L/S	Enroflox.
23	$2.2x10^6$	25	0.042	0.390	0.39	1.56	1.56	1.56	0.0485/0.097	0.390
24	$3.3x10^6$	50	0.021	0.195	0.39	3.12	0.78	1.56	0.0485/0.097	0.195
31	$3.3x10^6$	50	0.042	0.390	0.39	3.12	0.78	0.78	0.0485/0.097	0.195
32	$2.6x10^6$	50	0.042	0.390	0.39	25.00	6.25	1.56	0.097/0.195	0.390

According to the results all MS strains are resistant to oxytetracycline and sensitive to aivlosin, tylosin, tiamulin, lincomycin, lincomycin - spectinomycin combination (1:2) and enrofloxacin. These strains were sensitive to spectinomycin except isolate No.32 and semi-sensitive or resistant to erythromycin except isolate No. 23.

DISCUSSION

The purpose of the study was to determine the *in vitro* susceptibilities of *Mycoplasma gellisepticum* and *Mycoplasma synoviae*, which isolated from broiler chicken farms, Fars, Iran. *in vitro* susceptibility testing of mycoplasmas presents several problems that make standardization of methods difficult. Because no single medium is suitable for *in vitro* testing of all species, we had to use a different medium for each of the two species. It was recommended to add NAD to mediums for cultivating *Mycoplasma synoviea*. A pH indicator such as phenol red visualizes the pH shift due to biochemical activities of multiplying mycoplasmas. One of the major ways for *Mycoplasma gallisepticum* and *Mycoplasma synoviae* control is antibiotic therapy of affected flocks and infected hatching eggs (Alls *et al*., 1963). In Iran antibiotic administration has been accepted and different antibiotics are available and administered against mycoplasma infection for several decades. This is important to know that administration of antibiotics despite of dosage and duration of administration could not eradicate these organisms from the infected flocks. But antibiotics are able to reduce the severity of clinical signs and lesions, decrease egg production losses and other economic losses such as downgrading carcasses. Proper antibiotic therapy could significantly reduce population of *Mycoplasma gallisepticum* and *Mycoplasma synoviea* in the poultry respiratory tract.

Mycoplasma gallisepticum and *Mycoplasma synoviea* have shown sensitivity *in vitro* to several antimicrobials including tetracyclines, macrolides, lincosamides, fluoro-quinolones and others, but also are resistant to other antibiotics such as cephalosporins and penicillins which act by inhibiting cell wall biosynthesis. *Mycoplasma gallisepticum* may develop resistance and even demonstrate cross-resistance, to commonly used antibiotics (Bradbury *et al*., 1994; Zanella *et al*., 1998).

There appears to be no general agreement among mycoplasmologist about standard procedures for testing of mycoplasmas for sensitivity to antibiotics. Review of the published literatures describing sensitivity testing of mycoplasmas reveals that there is considerable various workers on this title. There appears to be no general agreement among mycoplasmologists about standard procedures for testing of mycoplasmas for sensitivity to antibiotics, for example, Lin had been used agar dilution method to determine susceptibility of avian mycoplasmas to antibiotics (Lin, 1987) Boughton also demonstrated tylosin susceptibility in 10 isolates of *Mycoplasma gallisepticum* tested by disc diffusion techniques (Boughton, 1982).

In this research the MICs were determined by a glucose metabolic inhibition method. Therefore, a micro-broth dilution procedure was chosen as the basic test design, because it was relatively simple and quick to perform and required only small volumes of media and other necessary materials such as different antibiotics. The test was also easy to read, since inhibition of mycoplasma growth by antibiotics could be deduced by the inability of the organism to ferment glucose and thus to produce a color change in a phenol red indicator in the medium from red to orange-yellow and generally more acceptable between mycoplasma scientists. As mentioned before, replicate doubling dilutions of antibiotics were made in 150 microliter volumes of mycoplasma broth in sterile 96-well U-bottomed micro titration plates (Hirose *et al*., 2003; Gautier-Bouchardon *et al*., 2002; Hannan *et al*., 1997a; Whithear *et al*., 1983) using 8-channel micropipette (Ependorff, Germany). The test was read when the phenol red indicator in the medium of a culture control (without antibiotic) had changed from deep red (pH 7.8) to orange-yellow (pH 7). It is believed that the number of viable mycoplasmas inoculated in each test of sensitivity to antibiotic was very important to reliability of the test. In this way to determine the concentration of each isolates in the mycoplasma broth and for making more accuracy the colony counting procedure was done (Hirose *et al*., 2003; Zanella *et al*., 1998). Some of other scientists preferred to determine the number of viable organisms by color changing unit (CCU) in the original culture by consulting the tables which were published by Meynell and Meynell (1970), (Hannan *et al*., 1997a,b; Whithear *et al*., 1983).

There is a wide range of bacteria and mycoplasma susceptibility to antibiotics in different area and countries. On the other hand another problem with

antibiogram test of mycoplasma is the different growth rate of any isolate to another one.

According to the results of our research, 28 isolates of *Mycoplasma gallisepticum* and four isolates of *Mycoplasma synoviea* showed various susceptibilities to the nine different antimicrobial agents which used in this research.

The breakpoints of MICs of each antimicrobial agent group: oxytetracycline, macrolides (aivlosin, tylosin and erythromycin), aminoglycoside (including spectinomycin), lincosamides (lincomycin), fluoroquinolone (enrofloxacin) and tiamulin were 1.0, 0.5-2.0, 2.0-4.0 and 1-2 μg/mL, 0.5-2.0 and 0.1-0.05 μg/mL, respectively (Hirose *et al*., 2003). When the MIC of the oxytetracycline was =1.0 μg/mL the isolate was considered susceptible, when the MIC was >1.0 μg/mL, the isolate was considered resistant.

In contrast to *Mycoplasma gallisepticum* isolates, all of *Mycoplasma synoviea* isolates have been shown resistance to oxytetracycline and almost erythromycin. This finding is in complete agreement with research results of Bradbury *et al*. (1994) and Whithear *et al*. (1983). Also, they used micro-broth dilution procedure for MIC detection in their research. The results also in agreement with reports of Kleven and Anderson (1971) and also Bradbury (1994) in the case of MICs of lincomycin-spectinomycin in comparison with oxytetracycline. Results of the present study agreed with those of Tanner and Wu (1992) who used broth micro-dilution test and showed *Mycoplasma gallisepticum* isolates were susceptible to lincomycin-spectinomycin and in the case of erythromycin and tylosin who found the susceptibility varied among their isolates that is similar to our findings. But they reported their isolates were sensitive to oxytetracycline. On the other hand Bradbury (1994) reported that tylosin had the highest activity followed closely by lincomycin, oxytetracycline, spectinomycin. Erythromycin was less effective, but like our findings lincomycin-spectinomycin had good activity against *Mycoplasma gallisepticum*.

According to our research aivlosin had greater activity during 9 antibiotics used with a range 0.012-0.042 μg/mL. The reason may be aivlosin is a newer antibiotic which is recently used in Iran against *Mycoplasma gallisepticum* and *Mycoplasma synoviea*. However, according to our knowledge the use of aivlosin in poultry is relatively limited in Europe and not at all in the USA. Comparison of MIC values with tylosin and tilmicosin would be interesting. Zanella *et al*. (1998) investigating development antibiotics resistance in *Mycoplasma gallisepticum*, also reported that cross-sensitivity tests using strains with induced resistance to the different antibiotics demonstrated that those which were resistant to tylosin were also resistant to other macrolides, where as strains made resistant to erythromycin appeared only less sensitive to tylosin. However, in our research the *Mycoplasma gallisepticum* and *Mycoplasma synoviea* isolates were more sensitive to tylosin than erythromycin.

Aivlosin, lincomycin-spectinomycin (1:2), tylosin, tiamulin, enrofloxacin and also lincomycin were commonly very effective in the field isolates. These findings are similar to the reports of Hannan (2000) and Zanella (1998). Although, macrolides and oxytetracycline were considered to be effective against mycoplasmas, these drugs (erythromycin and oxytetracycline) did not prove to be effective against *Mycoplasma synoviea* isolates. These MICs are higher than breakpoints of macrolides (0.5-0.2 μg/mL) or oxytetracycline (1 μg/mL). According to the commercial costs of used antibiotics against *Mycoplasma gallisepticum* and *Mycoplasma synoviea* tetracyclines such as oxytetracyline is not expensive and have been previously used in large scales to control and treat suspected broiler flocks to mycoplasma and *E. coli* infections and now observed that mycoplasma showed highly resistance to these kinds of antibiotics. On the other hand, aivlosin are more expensive then its use has limited. Also, aivlosin has been more recently introduced to producer. Also, our field experiences support these findings.

However, aivlosin was one of the most effective drugs against *Mycoplasma gallisepticum* and *Mycoplasma synoviea* as its MIC was the lowest of all oxytetracycline, erythromycin and spectinomycin were not effective against all isolates, as its MIC for some of isolates was extremely high. The MIC of erythromycin and oxytetracycline were distributed across a broad range. Resistant isolates to two mentioned antibiotics were obtained from the field.

Results showed that the sensitivity of isolates to spectinomycin and licomycin lonely was less than their sensitivity to lincomycin-spectinomycin in 1:2 combinations.

Sometimes the efficacy of various antibiotics towards *Mycoplasma gallisepticum* and *Mycoplasma synoviea* in the field is equal but some antibiotics show better advantages. Enrofloxacin still retains the acceptable advantage because it has broader spectrum of activity than the macrolides, being active also against a lot of gram-negative bacteria, which very often complicate *Mycoplasma gallisepticum* and *Mycoplasma synoviea* infections.

In some cases when an appropriate antibiotic, on the basis of antibiogram test, administrates the results may be out of our prediction and treatment results are not satisfy. It seems that some of avian respiratory viruses such as infectious bronchitis virus newcastle disease virus and pneumoviruses and also *E. coli* may interfere with *Mycoplasma gallisepticum* and *Mycoplasma synoviea* antibacterial treatments (Kleven, 1998a, b).

The treatment of these cases may be difficult because of

some viral coinfections such as infectious bronchitis, avian influenza or infectious bursal disease and then infection may remain for a long period. This finding is in agreement with other reports (Kleven and Anderson, 1971; Bradbury *et al*., 1994). Therefore, some of countries prefer to eradicate mycoplasma infections from their flocks by test and slaughter strategy.

Specimens in Iran: Ever since the establishment of the sensitivity to broad spectrum antibiotics (such as tetracyclines, macrolides and etc.) of *Mycoplasma gallisepticum* and *Mycoplasma synoviea* these and more recently even other antibiotics like tylosin and erythromycin have been widely used in both preventive and curative treatment of mycoplasma infection in poultry. This is true even when programs for the creation of mycoplasma-free flocks are applied in mycoplasma control and antibiotic treatment also is an important tool in the efforts to control mycoplasmosis by breaking the disease transmission chain via the hatching egg which is unanimously claimed to be the most important route of the infection.

Unfortunately despite of our review article, we could not find any report about mycoplasma antibiogram studies especially in poultry in the country. Now in Iran, the veterinary practitioners usually come to antibiotic treatment of mycoplasmal diseases of poultry on the basis of clinical signs of the affected poultries without any paraclinical findings and or antibiogram test before it. The results of this kind of blind antibiotic therapy are very harmful for human, poultry and life environment and also increase the costs.

The potential for mutation and for genetic exchange between all types of bacteria including mycoplasmas, combined with the short bacterial generation time, is of major importance in limiting the use of antimicrobial drugs in controlling infection in poultry. Sometimes, the blind use of antimicrobial drugs may does not induce resistance in bacteria but rather eliminates the susceptible bacteria and leaves the resistant bacteria already present in the population.

These above findings should be available to poultry disease specialists and veterinarians, as they consider therapeutic approaches to CRD and other poultry mycoplasmal diseases. Therefore, it is necessary to have a regular survey on poultry pathogenic mycoplasmas and to monitor antimicrobial susceptibility patterns in order to ensure that effective chemotherapy is being used to treat mycoplasmal infections.

However, mycoplasmal isolation, identification and antimicrobial susceptibility tests are essential and helpful for choosing an appropriate chemotherapy against CRD disease.

Conclusion: Whichever method of control is chosen, the need for effective antibiotics is apparent. According to our findings drug resistance must be taken into consideration in mycoplasma and this fact stresses the desirability for a choice between several antibiotics. The *in vitro* sensitivity of 28 different isolates of *Mycoplasma gallisepticum* and 4 isolate *Mycoplasma synoviea* have been tested using 9 different antibiotics including aivlosin and lincomycin-spectinomycin (1:2). These 2 antibiotics showed the highest effect followed by tylosin, enrofloxacin, tiamulin and lincomycin.

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