Indian Journal of Animal Sciences **89**(11): 1221–1223, November 2019/Short communication
https://doi.org/10.56093/ijans.v89i11.95864

Antibacterial susceptibility testing of *Mycoplasma gallisepticum* isolated from poultry

T S AHER[1], R S GANDGE[2] and S S BHAVE[3]

Bombay Veterinary College, Parel, Mumbai, Maharashtra 400 012 India

Received: 8 October 2018; Accepted: 4 April 2019

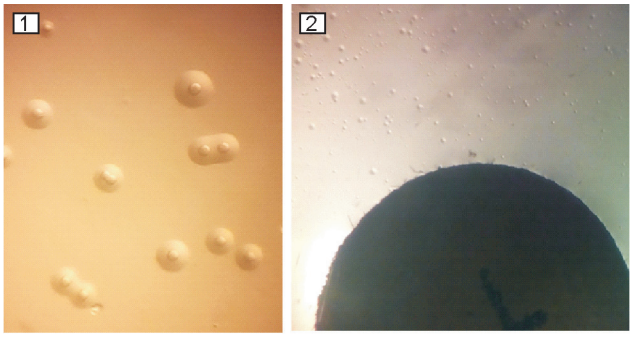
Key words: Antibacterial susceptibility, Disc diffusion, *Mycoplasma gallisepticum,* MIC, Poultry

M. gallisepticum (MG) is considered to be a major and the most important pathogen of poultry worldwide due to the great economic losses resulting from its infection. MG infection (CRD) causes poor feed conversion, low growth rate, increased mortalities, carcass condemnation, and increased vaccination and medication costs (Ley and Yoder 1997). In commercial layer flocks, MG is responsible for severe egg production losses (Domermuth and Gross 1962, Mohammed *et al.* 1987, Kleven 1998, Levisohn and Kleven 2000) and also it leads to deterioration of egg quality (Pruthi and Kharole 1981). In spite of the preventive programs, a lot of broiler and layer flocks are affected by MG during their production periods. The use of antimicrobials remains the most common and effective means for controlling mycoplasma infections in poultry farms. Although it is necessary to assess the sensitivity of mycoplasmas existing in the flock (Burch and Stipkovits 1994); most of the veterinarians prescribe antibiotics on the basis of clinical findings and their experiences for the treatment of affected flocks and improving egg production rate. Such indiscriminate use of therapeutics leads to decrease in the efficacy of antibiotics and development of resistance (Fahey 1957). Thus for a successful and aimed treatment of mycoplasma infection, regular antibiogram tests are necessary for monitoring susceptibility of *Mycoplasma gallisepticum* prevailing in the flock.

The choanal swabs were collected from CRD suspected birds (109) belonging to the semi-arid coastal area of Maharashtra (India) and processed for isolation of *Mycoplasma* spp. (OIE 2008). The positive cultures showing fried egg colonies (Fig. 1) were identified based on results of Diene's and Giemsa staining, Digitonine sensitivity test, Modified urease test and species specific 16SrRNA PCR (OIE 2008). MG isolates (25) comprising six isolates from present study and 19 archived field isolates from the Department of Microbiology recovered during 2013–15 were subjected to antibacterial susceptibility along with reference strain (ATCC 19610). Antibacterial susceptibility testing was carried out qualitatively by disc diffusion method as per Reda *et al*. (2012) using enrofloxacin (5 mcg), erythromycin (15 units), lincomycin (2 mcg), neomycin (15 mcg), oxytetracycline (30 mcg) and tylosin (15 mcg) (Himedia Lab. Pvt. Ltd). Minimum inhibitory concentrations (MICs) were determined by Microbroth dilution method as per Hannan (2000) using different concentrations of antibiotics, viz. enrofloxacin (0.012–0.4 µg/ml), oxytetracycline (0.780–5 µg/ml), tylosin (0.014–15 µg/ml), and neomycin (0.117– 60 µg/ml).

The optimum objectives of poultry producers are to improve the feed efficiency and productivity. However, the respiratory complications like chronic respiratory disease caused by MG along with other etiological agents lead to great economic losses to poultry industry. Therefore, the attempts for timely and targeted use of suitable antibacterial agent selected based on susceptibility testing will help to reduce the economic loss in poultry industry. The current study was mainly focused on four poultry farms from semi–arid and coastal areas of Maharashtra state of India.

Out of 109 specimens, six isolates of *M. gallisepticum* could be recovered with 5.55% prevalence rate. Out of 4 farms screened by cultural isolation, 3 (75%) were found positive for *M. gallisepticum* infection. Although, there are limitations in isolation of mycoplasma from clinical specimens, it has been considered as the 'gold standard' for diagnosis of mycoplasmosis. Though the procedure is grim, it is reliable and definitive since it provides direct evidence of presence of organism in the flock (OIE 2008)



Figs **1–2**. **1.** Colonies of *Mycoplasma gallisepticum* showing fried egg/ umbonate appearance under stereozoom microscope. **2**. *M. gallisepticum* showing resistance to lyncomycin in disc diffusion method (under sterieozoom microscope).

Present address: [1]MVSc scholar (drtrupti24@yahoo.com), [2]Associate Professor (rajashrigandge@yahoo.co.in),[3]Research Associate (sujata0bhave@gmail.com).

and its further characterization. The isolation rates of *M. gallisepticum* vary greatly due to a number of factors, viz. fastidious and fragile nature of organism, provision of suitable nutrients in proper proportion, over growth of other organisms, antibiotic therapy before collection of material, etc. Thus, variations in isolation rates of MG have been observed in the studies of various workers. The higher incidence rate of 55%, 47.23% and 14.85% of MG infection reported by Branton *et al.* (1984), Behbahan *et al.* (2005), and Hanif and Najeeb (2007) respectively. Whereas, lower incidence rate of 1.4% and 0% was reported by Ongar *et al.* (2009) and Nagalakshmi *et al.* (2013) respectively. However, Tiong *et al.* (1979) observed 5.5–8.8% isolation rate of MG, closer to the rate of present investigation.

Mycoplasmas have very small genome size; they fail to show many biochemical pathways as compared to other bacteria for identification at species level conventionally**.** Therefore to overcome this difficulty, 16S rRNA PCR is recommended (OIE 2008). In 16S rRNA PCR, all six isolates including reference strain yielded an amplification product of 185 bp. According to Behbahn *et al.* (2005), Gharaibeh *et al.* (2008) and Kaboli *et al.* (2013), PCR is very useful tool for identification of MG.

In *in vitro* antibiotic susceptibility testing of MG isolates by disc diffusion method, all 25 (100%) isolates were resistant to lincomycin, 13 (52%) to erythromycin and 9 (36%) to Neomycin. On the contrary, all (100%) isolates were highly sensitive to tylosin and moderately sensitive to oxytetracycline and enrofloxacin (Figs 2, 3). Since the use of live mycoplasma vaccines is not widely allowed in many countries; the use of antimicrobials remains the most common and effective means for controlling mycoplasma infections in poultry farms. Disc diffusion method is most suitable and easy technique for selecting the effective antibiotic for therapeutic purpose and epidemiological study. In the present study, the highest resistance was observed to lyncomycin followed by erythromycin and neomycin. Conversely, the highest sensitivity was displayed to tylosin, whereas moderate sensitivity was observed to enrofloxacin and oxytetracycline. Sensitivity pattern of isolates varied with geographical area, thus isolates from diverse origin differed in sensitivity pattern. Whereas, similar antibiogram pattern of isolates originated within same geographical area indicated the circulation of same strains in that particular geographical area. Thus, in addition to selection of antibiotic and elimination of resistant antibiotic in therapy; the antibiogram study also helps to know the epidemiology of MG infection. Sensitivity pattern to tylosin, neomycin and erythromycin observed in our study was similar to the results reported by Rada *et al.* (2012). However, due to scanty data available on disc diffusion assay of MG, we felt limitation in comparative analysis and critical discussion of the results of the present study.

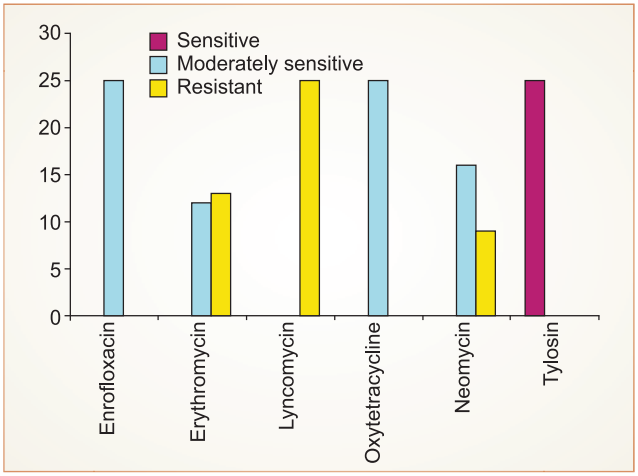


Fig. 3. Antibiogram pattern of *M. gallisepticum*

Minimum inhibitory concentration (MIC) of four antibacterial agents was determined against 25 MG field isolates. Among the antibacterial agents tested, neomycin showed highest MIC value, whereas tylosin had lowest MIC value against *M. gallisepticum* isolates. However, the MIC value of ATCC strain and field isolates differed (Table 1).

Table 1. MIC values of antibacterial agents against *M. gallisepticum*

Antibiotics	MIC (µg/ml)	
	Field isolates	ATCC strain
Enrofloxacin	0.15 – 0.625	0.312
Oxytetracycline	0.468 - 0.973	0.937
Neomycin	0.468 – 1.87	0.468
Tylosin	0.11 – 0.23	0.010

Minimum inhibitory concentration of tylosin, enrofloxacin, oxytetracycline and neomycin was determined by microbroth dilution in 25 field isolates of *Mycoplasma gallisepticum.* All antibiotics yielded different range of MIC values which varied with type of the isolates and antibiotics used. Tylosin (0.11–0.23 µg/ml) showed lowest MIC value followed by enrofloxacin (0.15–0.625 µg/ml), oxytetracycline (0.468–0.973 µg/ml) and neomycin (0.468–1.87 µg/ml). In our study, MIC value of tylosin determined by microbroth dilution method was comparable with MIC value previously reported by Jordan *et al.* (1998) and Bradbury *et al.* (1994). Whereas, comparatively lower MIC value of tylosin was observed by Tanner and Wu (1992). The MIC of oxytetracycline and enrofloxacin observed in present study was similar to MICs reported by Bradbury (2007) and Wang *et al.* (2001). The results of susceptibility testing of MG against antimicrobial agents showed tylosin as most effective over other agents. Similar results of *in vivo* and *in vitro* efficacy of tylosin was reported by Farran *et al.* (2018) and Khatoon *et al.* (2018) respectively.

SUMMARY

This is the first study on antibacterial susceptibility of *M. gallisepticum* in Maharashtra state of India. Since the use of live mycoplasma vaccines is not widely allowed in the country; the use of antimicrobials remains the most

common and effective means for controlling mycoplasma infections in poultry farms. Therefore, the current study aimed to study emergence of resistance in MG to antibacterial agents and to find out the effective antibacterial agent for treatment of CRD. In disc diffusion method, all 25 *M. gallisepticum* isolates were highly sensitive to tylosin followed by enrofloxacin and oxytetracycline. Till date, tylosin was the most effective therapeutic agent at lowest concentration for treatment of CRD. However, higher MIC values of isolates than ATCC strain are indication of initiation of emergence of antibacterial drug resistance in *M. gallisepticum*. Therefore, it is advised to select effective antibiotic based on the result of susceptibility test for the treatment of CRD to prevent further emergence of drug resistance.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge and thank Dr. Siddharth Sable, Livestock Development Officer, Palghar, Maharashtra for kind cooperation for collection of clinical specimens.

REFERENCES

Behbahan G, Asasi K, Afsharffar A R and Pourbakhsh S A. 2005. Isolation and identification of *Mycoplasma gallisepticum* by PCR and RFLP. *Iranian Journal of Veterinary Research* **6**: 2.

Bradbury J M. 2007. Biosecurity and vaccination control *Mycoplasma* infections. *World Poultry* **23**: 35–36.

Bradbury J M, Yavari C A and Giles C G. 1994. *In vitro* evaluation of various antimicrobials against *Mycoplasma gallisepticum* and *Mycoplasma synoviae* by the micro-broth method and comparison with a commercially prepared test system. *Avian Pathology* **23**: 105–15.

Branton S L, Gerlach H and Kleven S H. 1984. *Mycoplasma gallisepticum* isolation in layers. *Poultry Science Journal* **63**: 1917–19.

Burch D G S and Stipkovits L. 1994. Efficacy of tetramutin premix in the prevention of an artificial infection with *Mycoplasma gallisepticum* in broilers in the presence of SAL. Proceedings of the 9th European Poultry Conference, Glasgow, UK.

Domermuth C H and Gross W B. 1962. The production of salpingitis of chickens by *Mycoplasma gallisepticum*. *Avian Diseases* **6**: 499–505.

Fahey J E. 1957. Infectious sinusitis of turkeys caused by an antibiotic resistant pleuropneumonia-like organism. *Veterinary Medicine* **52**: 305–07.

Farran M T, Ellakany H F, Shaib H A and Majed H M. 2018. Evaluation of antibiotics to control *Mycoplasma gallisepticum* in broiler breeder chickens. *Poultry Fisheries Wildlife Sciences* **6**: 1–6.

Gharaibeh S and Roussan D A. 2008. The use of molecular techniques in isolation and characterization of *Mycoplasma gallisepticum* from commercial chickens in Jordan. *International Journal of Poultry Science* **7**: 28–35.

Hanif A and Najeeb M I. 2007. Comparison of conventional bacterial isolation, rapid slide agglutination and polymerase chain reaction for detection of *Mycoplasma gallisepticum* in breeder flocks. *Pakistan Journal of Life Sciences* **5**: 1–5.

Hannan P C T. 2000. Guidelines and recommendations for antimicrobial minimum inhibitory concentration (MIC) testing against veterinary mycoplasma species. *Veterinary Research* **31**: 373–95.

Jordan F T W, Forrester C A, Ripley P H and Burch D G. 1998. *In vitro* and *in vivo* comparisons of valnemulin, tiamulin, tylosin, enrofloxacin and lincomycin/spectino mycin against *Mycoplasma gallisepticum*. *Avian Diseases* **42**: 738–45.

Kaboli K P, Bijanzad A R, Moggadam J, Shahbazi M and Hosseini H. 2013. Evaluation of *Mycoplasma gallisepticum* infection diagnosis in rural poultry by 16S rRNA PCR methods. *European Journal of Zoological Research* **2**: 63–66.

Khatoon H, Afzal F, Tahir M F, Hussain M and Khan S U. 2018. Prevalence of mycoplasmosis and antibiotic susceptibility of *Mycoplasma gallisepticum* in commercial chicken flocks of Rawalpindi Division, Pakistan. *Pakistan Veterinary Journal*. DOI: 10.29261/pakvetj/2018.081.

Kleven S H. 1998. *Mycoplasma* in the etiology of multifactorial respiratory diseases. *Poultry Science* **77**: 1146–49.

Levisohn S and Kleven S H. 2000. Avian mycoplasmosis (*Mycoplasma gallisepticum*). *Scientific Technical Review Office of International des Epizooties* **19**: 425–42.

Ley D H and Yoder H W. 1997. *Mycoplasma gallisepticum* infection. pp. 194–207. (Ed.) Calnek B W. *Diseases of Poultry.* 10th edn. Iowa State University Press, Ames, Iowa, USA.

Mohammed H O, Tim E C and Yamamoto R. 1987. Economic impact of *Mycoplasma gallisepticum* and *M. synoviae* in commercial layer flocks. *Avian Diseases* **31**: 477–82.

Nagalakshmi K S, Senthil Kumar T M A, Parthiban M and Ramadass P. 2013. Differential diagnosis of avian mycoplasmosis. *Indian Veterinary Journal* **90**: 12–15.

OIE. 2008. Avian mycoplasmosis (*Mycoplasma gallisepticum, M. synoviae*). Chapter 2.3.5. OIE Terrestrial Manual, pp. 482–96.

Ongor H, Kalin R, Karahan M, Çetinkaya B and Akan M. 2009. Detection of mycoplasma species in turkeys by culture and polymerase chain reaction. *Scientific Technical Review Office of International des Epizooties* **28**: 1103–09.

Pruthi A K and Kharole M U. 1981. Sequential pathology of genital tract in chickens experimentally infected with *Mycoplasma gallisepticum*. *Avian Diseases* **25**: 768–78.

Reda L M, Abd E and Samie L K. 2012. Some studies on the diagnosis of *Mycoplasma gallisepticum* in chicken. *Nature Science* **10**: 247–51.

Tanner A C and Wu C C. 1992. Adaptation of sensititre broth microdilution technique to susceptibility testing of *Mycoplasma gallisepticum. Avian Diseases* **36**: 714–17.

Tiong S K, Liow T M and Tan R J S. 1979. Isolation and identification of avian mycoplasmas in Singapore. *British Poultry Science* **20**: 45–54.

Wang C, Ewing M and A'arabi S Y. 2001. *In vitro* susceptibility of avian mycoplasmas to enrofloxacin, sarafloxacin, tylosin, and oxytetracycline. *Avian Diseases* **45**: 456–60.