Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.5886

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **5 (5):** 1329-1334 (2017)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

In-Vitro Antimicrobial Sensitivity of Avian Mycoplasmas Isolated from Broiler Chicken Flocks Affected with Respiratory Infections

Piyush Tomar*, Y. Singh, N. K. Mahajan and N. Jindal

Department of Veterinary Public Health and Epidemiology, College of Veterinary Sciences

Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125 004, Haryana

*Corresponding Author E-mail: tomarvety@gmail.com

Received: 15.09.2017 | Revised: 17.10.2017 | Accepted: 20.10.2017

ABSTRACT

Avian mycoplasmosis is one of the major problems facing the poultry industry, all over the world. Mycoplamsa gallisepticum (MG) and Mycoplasma synoviae (MS) are the two most pathogenic avian mycoplasmas. The present study describes the antibiogram pattern of 13 mollicutes including Mycoplamsa gallisepticum (8), Mycoplasma synoviae (1), Mycoplasma gallinarum (3) and Acholeplasma laidlawii (1) against 21 different antibiotics. These mycoplasma strains were retrieved from the Department of Veterinary Public Health and Epidemiology, LUVAS Hisar which were previously isolated from poultry showing respiratory infection and the in vitro single disc diffusion technique was employed using Frey's agar medium. Mollicutes were found sensitive to amikacin, enrofloxacin, tylosin, spiromycin, chloramphenicol, chlortetracycline and norfloxacin whereas resistant to cloxacillin, nalidixic acid, ceftriaxone, cefotaxime, cefoperazone, and ampicillin. The colistin, lincomycin, levofloxacin, erythromycin,ciprofloxacin, streptomycin, gentamicin and oxytetracycline showed variable results which formed the basis to formulate resistotype pattern of Mycoplasma gallisepticum and M. synoviae the well known pathogens. It has been concluded from this study that frequent surveillance of antibiotic sensitivity against avian mycoplasmosis should be undertaken to adopt a suitable control measure on the poultry farms of Haryana.

Key words: *Avian mycoplasmas, antibiogram, Mycoplamsa gallisepticum, Mycoplasma synoviae, Acholeplasma laidlawii and Mycoplasma gallinarum*

INTRODUCTION

The fastest growing segments of the animal husbandry in Haryana are poultry industry. Respiratory complications are the main cause of econonomic losses in poultry due to increased mortality resulting from. Mycoplasma infection is one of the most important causes of respiratory infection producing great economic losses to poultry production. So far, 25 species of mycoplasma have been reported from birds.

Cite this article: Tomar, P., Singh, Y., Mahajan, N.K. and Jindal, N., *In-Vitro* Antimicrobial Sensitivity of Avian Mycoplasmas Isolated from Broiler Chicken Flocks Affected with Respiratory Infections, *Int. J. Pure App. Biosci.* **5(5):** 1329-1334 (2017). doi: http://dx.doi.org/10.18782/2320-7051.5886

The *Mycoplasma gallisepticum* and *Mycoplasma synoviae* has long been recognized as common respiratory pathogen especially in chickens causing lots of commercial losses in poultry industries. *M. gallisepticum* commonly induces chronic respiratory disease in chicken[18]. *Mycoplasma gallisepticum* infection has tropism primarily for mucosal membranes of respiratory tract, conjunctiva and sinuses[8] where upper airways and trachea are the preferred sites of infection for most of strains. Economic losses due to MG occurs due to reduction in egg production, an increase in embryo mortality and chick mortality and a reduction in weight gain and feed conversion efficiency[6]. *M. synoviae* most frequently occurs as a subclinical upper respiratory infection but may result in air sacculitis and synovitis in chicken and turkeys[7]. The *M. gallinarum* and *A. laidlawii* are usually considered as non-pathogenic commensal but sometime serve as a cofactor with pathogenic poultry viruses including Newcastle disease virus and infectious bronchitis virus[4] . As the use of vaccination programme for the control of mycoplasmas is very limited in Haryana, and most often chemotherapy in association with biosecurity measures is practiced to minimize the economic losses and to avoid lateral and vertical transmission. For this purpose various group of antibiotics including macrolides, tetracyclines and flouroquinolones are being used which induce either DNA fragmentation or inhibition of protein synthesis and are the drugs of choice for treatment against mycoplasmosis but mycoplasmas are resistant to penicillin because of lack of cell-wall and are not used for their treatment[2,3,13]. Most of the veterinarian prescribes these antibiotics on the basis of clinical finding and their experience in order to treat the affected flocks or supplemented in feed by the farmers to improve egg production which led to the development of antimicrobial resistance in avian mycoplasmas over the period of their usage. This paper describes the study on the *in-vitro* antimicrobial sensitivity pattern of avian mycoplasmas isolated from poultry affected with respiratory infection.

MATERIAL AND METHODS

The present study describes the antibiotic sensitivity of 13 mollicutes including 8 *Mycoplamsa gallisepticum*, one *Mycoplasma synoviae*, 3 *Mycoplasma gallinarum* and one *Acholeplasma laidlawii* against 21 different antibiotics. These mycoplasma strains were retrieved from the Department of Veterinary Public Health and Epidemiology, LUVAS Hisar for performing the test and were maintained in Frey's broth and agar media. These strains were isolated previously from poultry flocks affected with respiratory infections. For performing antimicrobial sensitivity test *in vitro* single disc diffusion technique[1] was used using Frey's agar medium and various antibiotic discs viz., ampicillin (25 mcg), cefaperozone (30 mcg), amikacin (10 mcg), cefotaxime (10 mcg), ceftriaxone (10 mcg), chlortetracyclin (30 mcg), chloramphenicol (10 mcg), ciprofloxacin (30 mcg), cloxacillin (10 mcg), enrofloxacin (10 mcg), colistin (50 mcg), erythromycin (10 mcg), gentamicin (50 mcg), nincomycin (15 mcg), levofloxacin (5 mcg), nalidixic acid (50 mcg), norfloxacin (5 mcg), oxytetracyclin (30 mcg), streptomycin (10 mcg), spiramycin (30 mcg), tylosin (15 mcg) were used. On the basis of inhibition zone the results were interpreted as per the manufacturer's recommendations (Hi-Media, Mumbai) after 5-days incubation at 37°C in candle jar.

RESULTS AND DISCUSSION

Until this study information about the susceptibility of avian mycoplasmas isolates of Haryana to antimicrobials is scarce. The objective of this study was to determine the *in vitro* antibiotic susceptibilities of avian mycoplasmas, which were isolated earlier from broiler chicken flocks affected with respiratory infections. In present study Frey's broth and Frey's agar medium with penicillin were used to carry out antibiotic sensitivity testing of mycoplasmas. As mycoplasmas do not have cell wall and are resistance to penicillin. Thus, penicillin antibiotic has no effect on mycoplasmas and it doesn't interfere in determining *in vitro* antibiotic sensitivity of mycoplasmas[14]. In the present study, the

antibiotic sensitivity of 13 mollicutes including *Mycoplamsa gallisepticum* (8), *Mycoplasma synoviae* (1), *Mycoplasma gallinarum* (3) and *Acholeplasma laidlawii* (1) against 21 different antibiotics were recorded. It was found in this study that all the isolates were found sensitive to 7 antibiotics such as amikacin, chloramphenicol, enrofloxacin, tylosin, spiromycin, norfloxacin and chlortetracycline. Reda and Abd[9] also reported, sensitivity of MG isolates to tylosin, and enrofloxacin which is in partial agreement to our study (Table 1).

All the isolates were found resistant to 6 antibiotics such as nalidixic acid, cloxacillin, ceftriaxone, cefotaxime, cefoperazone, and ampicillin. Valks and Burch[10] also reported that cloxacillin and lincomycin resistance increased in the last few decades.

Eight antibiotics *viz.*, erythromycin, colistin, levofloxacin, lincomycin, ciprofloxacin, streptomycin, gentamicin and oxytetracycline exhibited variable results which formed the basis to formulate resistotype pattern of *Mycoplasma gallisepticum* and *M. synoviae* strains. Amongst eight strains of *Mycoplasma gallisepticum* and one strain of *M. synoviae* the four resistotype could be detected (Table 2). One strain showed resistotype R-1 and R-2 in each *viz.*, PT-29 and PT-36 respectively showing 11.11 per cent relative frequency whereas resistotype R-3 represented two strains (PT-78 and PT-35) with 22.22 per cent relative frequency. The R-4 was the most frequently occurring resistotype which represented five strains with relative frequency of 55.55 per cent showing complete resistance to two antibiotics viz. colistin and oxytetracycline. For epidemiological study this most frequently occurring resistotype may form the basis as marker. Kapoor[5] also shown the importance of most frequently occurring resistotype as a epidemiological marker. Oxytetracycline showed resitance to 8 mycoplasma isolates (PT-1, PT-20, PT-36, PT-47, PT-71, PT-72, PT-80, and PT-90). This resistance to oxytetracycline was possibly due to its frequent usage for a longer period on indian poultry farms. Sensitivity to Tetracyclines is not present sometimes and is common which may be due to acquisition of the *tetM* gene[16] . In present study, Macrolides such as erythromycin were also showing resistance in 3 isolates (PT-4, PT-20, and PT-36) which is supported by the findings of Wu *et al*[13] who reported a high resistance to erythromycin. The resistance of mycoplasmas to erythromycin may be probably due to mutations in the domain V loop of the 23S rRNA gene, leading to a reduction in the affinity of macrolides to ribosome[11,12,13]. Most of the antibiotics to which mycoplasma are susceptible they only inhibit their multiplication and do not kill them. Mycoplasmas are also innately resistant to some other antibiotics such as rifampicins[17]. They may develop resistance, either by acquisition of a resistance gene or by gene mutation, to antibiotics to which they are usually sensitive. The antibiotic susceptibility pattern are also influenced by the source of the mycoplasma viz., isolate recovered from a eukaryotic cell line which was contaminated that has been subjected to various antibiotic treatment may have an different antibiotic profile from the same mycoplasmal species that has been isolated directly from animal source[17] . Mycoplasmas are difficult to eradicate from animal hosts or from cell cultures by antibiotic treatment because of developed resistance to the routine antibiotics used and also due to the fact that some mycoplasmas invade inside the eukaryotic cells[17]. Most of the antibiotics unable to kill mycoplasmas, despite the fact that they may suppress their growth, is one of the reasons why eradication from the host tissues is often slow[15]. Eradication is also difficult in immunosuppressed or immunodeficient poultry.

In present study, mycoplasmas strains were at first or second passage level, thus there is no possibility that the resistance developed during the microbial passages. The history of antibiotics used in poultry farms of Haryana indicated that only those antibiotics are showing resistance which are routinely used in the prevention program. In poultry farms of Haryana, chicken growers/growth promoters and antibiotics are frequently used in feed and

water of broilers and layers to reduce the MG infection. This could be the reason that in these areas the mycoplasma strains are possibly resistant to most antibiotics that were previously used as prophylactic measures. In this study due to limited information, it is difficult to compare levels of antibiotic resistance in different geographical locations. In conclusion our study showed that regular surveillance of antibiotic sensitivity against MG should be done to adopt control programme in the poultry farms of Haryana.

Table 1: Antibiotic sensitivity pattern of field strains of *Mycoplasma* and *Acholeplasma* species isolated from chicken affected with respiratory infection

Antibiotic discs	Laboratory number of field isolates												
	PT-1	PT-4	PT-17	PT-20	PT-29	PT-35	PT-36	PT-47	PT-71	PT-72	PT-78	PT-80	PT-90
	A. laidlawii	*M. gallinarum*			*M. synoviae*	*M. gallisepticum*							
Amikacin (10 mcg)	S	S	S	S	S	S	S	S	S	S	S	S	S
Ampicillin (25 mcg)	R	R	R	R	R	R	R	R	R	R	R	R	R
Cefaperozone (30 mcg)	R	R	R	R	R	R	R	R	R	R	R	R	R
Cefotaxime (10 mcg)	R	R	R	R	R	R	R	R	R	R	R	R	R
Ceftriaxone (10 mcg)	R	R	R	R	R	R	R	R	R	R	R	R	R
Chloramphenicol(10mcg)	S	S	S	S	S	S	S	S	S	S	S	S	S
Chlortetracyclin (30 mcg)	S	S	S	S	S	S	S	S	S	S	S	S	S
Ciprofloxacin (30 mcg)	R	S	R	S	S	R	S	S	S	S	R	S	S
Cloxacillin (10 mcg)	R	R	R	R	R	R	R	R	R	R	R	R	R
Colistin (50 mcg)	S	S	S	R	R	R	S	S	S	S	R	S	S
Enrofloxacin (10 mcg)	S	S	S	S	S	S	S	S	S	S	S	S	S
Erythromycin (10 mcg)	S	R	S	R	S	S	R	S	S	S	S	S	S
Gentamicin (50 mcg)	S	S	S	S	S	S	R	S	S	S	S	S	S
Levofloxacin (5 mcg)	S	R	S	S	S	S	S	S	S	S	S	S	S
Lincomycin (15 mcg)	R	S	S	R	S	S	S	S	S	S	S	S	S
Nalidixic acid (50 mcg)	R	R	R	R	R	R	R	R	R	R	R	R	R
Norfloxacin (5 mcg)	S	S	S	S	S	S	S	S	S	S	S	S	S
Oxytetracyclin (30 mcg)	R	S	S	R	S	S	R	R	R	R	S	R	R
Spiramycin (30 mcg)	S	S	S	S	S	S	S	S	S	S	S	S	S
Streptomycin (10 mcg)	R	S	R	R	S	S	R	R	R	R	S	R	R
Tylosin (15 mcg)	S	S	S	S	S	S	S	S	S	S	S	S	S

S = sensitive, R = resistant

Table 2: Resistotype pattern of field strains of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* species isolated from chicken affected with respiratory infection

Resistotype	Sensitivity or resistant to antibiotics discs								No. of isolates (Lab no.)	Per cent relative frequency
	C	Cl	E	ER	G	L	LI	O		
R-1	S	R	S	S	S	S	S	S	1 (PT-29)	11.11
R-2	S	S	S	R	R	S	S	S	1 (PT-36)	11.11
R-3	R	R	R	S	S	S	S	S	2 (PT-35, PT-78)	22.22
R-4	S	R	S	S	S	S	S	R	5 (PT-47, PT-71, PT-72,PT-80, PT- 90)	55.55

C = Ciprofloxacin (30 mcg), CL = Colistin (50 mcg), E = Enrofloxacin (10 mcg), ER = Erythromycin (10 mcg), G = Gentamicin (50mcg), L = Levofloxacin (5 mcg), LI = Lincomycin (15 mcg), O = Oxytetracyclin (30 mcg)

Acknowledgement

The authors are thankful to Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

Authors would hereby declare that there is no conflict of interests.

REFERENCES

1. Bauer, A.W., Kirby, W.M.M., Sherris J.C. and Turck, M., Antibiotic susceptibility testing by a standardized single disk methd, *Anm J Clin Pathol*. **45**: 493-496 (1966).
2. Bébéar, M., Renaudin, J. and Charron, A., Mutations in the gyrA, parC, and parE genes associated with fluoroquinolone resistance inclinical isolates of *Mycoplasma hominis*, *Antimicrob Agent Chemother*, **43**: 954–956 (1999).
3. Bradbury, J.M., Abdul, Wahab O.M.S., Yavari, C.A., Dupiellet, J.P. and Bove, J.M., *Mycoplasma imitans* sp. nov. is related to *Mycoplasma gallisepticum* and found in birds, *Int J Syst Bacteriol*, **43**: 721-728 (1993).
4. Bradbury, J.M., Avian mycoplasma infections: prototype of mixed infections with mycoplasmas, bacteria and viruses, *Ann. Microbiol*. (Paris). **135A**: 83-89 (1984).
5. Kapoor, PK., Experimental epidemiological studies on Mycoplasma mycoides subsp. mycoides (Large Colony Type) isolated from natural bovine abortions. Ph.D. thesis submitted to CCS HAU, Hisar (India) (1993).
6. Kleven, S.H., Summary of discussions of avain mycoplasma teaminternational researh program on comparative Mycoplasmology, Hungary October 16–20, 1989, *Avian Pathol*. **19**:4 795-800 (1990).
7. Kleven, S.H., *Mycoplasma synoviae* infection. In: *Diseases of poultry*, Edited by Y.M. Saif 11 th ed. AAAP, Iowa State Press,756- 66 (2003).
8. Levisohn, S. and Kleven, S.H., Avian mycoplasmosis (*Mycoplasma gallisepticum*), *Rev. sci. tech. Off. int. Epiz*. **19**: 425-444 (2000).
9. Reda, L.M. and Abd, E.L. L.K., Some studies on the diagnosis of Mycoplasma gallisepticum in chicken, Nature and science. **10**: 247-251 (2012).
10. Valks, M. and Burch, D.G.S., Comparative activity and resistance development of tiamulin and other antimicrobials against avian Mycoplasma. Presenatation at world veterinary poultry association, cairo 200 (2002).
11. Lucier, T.S, Heitzman, K., Liu, S.K and Hu, P.C., Transition mutations in the 23S rRNA of erythromycin-resistant isolates of Mycoplasma pneumonia, Antimicrob. Agents Chemother. **39**: 2770–2773 (1995).
12. Gautier-Bouchardon, A.V, Reinhardt, A.K, Kobisch, M. and Kempf., In vitro development of resistance to enrofloxacin, erythromycin, tylosin, tiamulin and oxytetracycline in Mycoplasma gallisepticum, Mycoplasma iowae and Mycoplasma synoviae. *Vet. Microbiol* **88**: 47–58. (2002).
13. Wu, C.M., Wu, H., Ning, Y., Wang, J., Du, X and Shen, J., Induction of macrolide resistance in Mycoplasma gallisepticum in vitro and its resistance-related mutations within domain Vof 23S rRNA., *FEMS Microbiol*. Lett. **247**: 199–205. (2005).
14. Whithear, K.G, Bowtell, D.D, Ghiocas, E and Hughes, K.L., Evaluation and use of a micro-broth dilution procedure for testing sensitivity., *Avian Dis*. **27**: 937–949. (1983).
15. Renaudin, H, Aydin, M.D and Bébéar, C., Ketolides and mycoplasmas: *in vitro* evaluation of RU 004. In Program and Abstracts of the Thirty-Fifth Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco CA, Abstract *American Society for Microbiology,* Washington,DC.**168:** 142 (1995).

16. Roberts, M.C, Koutsky, L.A, Holmes, K.K, LeBlanc, D.L and Kenny, G.E., Tetracycline-resistant *Mycoplasma hominis* strains contain streptococcal *tetM* sequences., *Antimicrob. Agents Chemo.* **28**: 141–143 (1985).
17. Taylor-Robinson David and Bébéar, Christiane., Antibiotic susceptibilities of mycoplasmas and treatment of mycoplasmal infections, *J. Antimicrob. Chemother*.. **40:** 622–630 (1997).
18. Ley, D.H., *Mycoplasma gallisepticm* infection. In Saif, Y.M, Barnes, H.J., Glisson, J.R., Fadly, A.M., McDougald, L.R and Swayne, D.E., Ed. Disease of Poultry. Iowa state University Ress, Ames, Iowa, USA, 722-744 (2003).