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Minimum inhibition concentration of two antibiotics and two plants extract against *Mycoplasma galisepticum* in Iraq

Ansam Khalid Mohammed and Dalia Abd-Al-Kareem Abd-Al-Shaheed
Department of Microbiology / College of Veterinary Medicine / University of Baghdad, Iraq.

Abstract:
This study was designed in order to evaluate the effectives of two antibiotics and two plant extracts against Mycoplasma galisepticum. Two commercial antibiotics tilmicosin and enrofloxacin used for minimum inhibition concentration detection against Mycoplasma galisepticum isolates. Two plants extracts also used for the same purpose which is garlic and thyme. Using the micro-broth technique to determine the growth of in vitro activities of tilmicosin, enrofloxacin, garlic and thyme against the isolates of Mycoplasma gallisepticum. The results showed that the tilmicosin had MICs of 7.8 mg/ml. while, enrofloxacin showed the best in vitro activity giving MICs of 1.56 mg/ml. Garlic had MICs of 0.97 mg/ml which was the best of all results. Lastly thyme had MICs of 15.6 mg/ml for the Mycoplasma gallisepticum. According to the results all Mycoplasma gallisepticum strains are sensitive to all antibiotics and plants extracts in different level and the highest effectiveness was for the garlic.
Keyword: *Mycoplasma galisepticum, MIC, antibiotics, extract, Iraq.*

Introduction:

Mycoplasma galisepticum (MG) is the most pathogenic and economically significant mycoplasma pathogen of poultry. MG economically affects the poultry industry through increased mortality and decreased egg production (Carpenter et al., 1981) and reduced feed efficiency (Almanama, 2011). Some researchers demonstrated that MG infected flocks produced 5-12 fewer eggs per chicken hen compared with uninfected flocks (Ley, 2003). Airsacculitis in chickens can cause significant condemnations at slaughter. Increased medication costs are additional factors that make this disease one of the costliest disease problems for poultry industry (Almanama, 2011). *Mycoplasma gallisepticum* is the mainly pathogenic avian Mycoplasma species with significant economic cause as one of the costliest diseases confront poultry industry worldwide because of a decrease in egg production and egg quality, low hatchability (Peebles and Branton, 2012). Decline of feed efficiency, increase in mortality and carcass condemnations, in addition costs of control (including medication and vaccination) and prevention (including surveillance, biosecurity and eradication) (Levisohn and Kleven, 2000; Nascimento *et al.*, 2005).

Antibodies persisted in recovered chickens, and upon re-exposure, they had a faster rate of MG elimination and less severe tracheal lesions than observed after the first exposure. The importance of antibodies produced in response to MG infection inhibited attachment of the organism to epithelial cells (Grodio *et al.*, 2009). Mycoplasmas may be difficult to eradicate from human or animal hosts or from cell cultures by antimicrobial treatment for a number of reasons. This may be a result of intrinsic or acquired resistance to the antimicrobial, a lack of cidal activity, or because there is invasion of eukaryotic cells by some mycoplasmas (Taylor-Robinson and Bebear, 1997). With the increase in resistance of many microorganisms to the currently used antimicrobials and the high cost of production of synthetic compounds; in addition to many side effects; there is a need to look for alternatives. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties (Marjorie, 1999). Plants have provided a good source of antiinfective agents; emetine, quinine, and berberine remain highly effective instruments in the fight against microbial infections (Marjorie, 1999). Plants containing protoberberines and related alkaloids, picralima-type indole alkaloids and garcinia biflavonones used in traditional African system of medicine, have also been found to be active against a wide variety of micro-organisms. Phytomedicines derived from plants have shown great promise in the treatment of intractable infectious diseases including opportunistic AIDS infections (Ahmad *et al.*, 1996). People on all continents have long applied poultices and imbibed infusions of hundreds of indigenous plants, dating back to prehistory. These plants are still widely used in ethno-medicine around the world. Historically, therapeutic results have been mixed although they often resulted in cures or relief of symptoms (Stockwell, 1988). The use of plant extracts, as well as other alternative forms of medical treatments, has been enjoying great popularity since the late 1990s. Approximately one-third of people surveyed in the United States used at least one "unconventional" therapy (Eisenberg *et al.*, 1993). A relatively small percentage 1-

10% of plants on The Earth is used as foods by both humans and other animal species. It is possible that even more are used for medicinal purposes (Moerman, 1996). It is known to have more than 2000 plant species belonging to 700 genera (Afifi and Abu-Irmaileh, 2000). Among these plants, as many as 485 species from 99 plant families are categorised as medicinal plants (Oran and Al-Eisawi, 1998). The high cost of synthetic antimicrobials, the side effects caused by these antimicrobials and the development of drug resistance by microorganisms against commonly used antimicrobials requires that new and alternative antimicrobial substances are investigated. Medicinal plants contain a variety of compounds that are used for treating chronic and infectious diseases by treating and/or protecting the susceptible humans and/or animals to such diseases. Medicinal plants could therefore offer alternatives treatments to the currently used antimicrobials (Al-Momani *et al.*, 2007). Use of antibiotic that might result in deposition of residues in meat and eggs must not be permitted in food intended for human consumption. If use of antibiotics is necessary as in prevention and treatment of animal diseases, a withholding period must be observed until the residues are negligible or no longer detected (Nisha, 2008). There were little published data about mycoplasmosis infection in Iraq Jameel and Hasso (2018) and Ali (2019), so this study was designed in order to increase knowledge about this disease.

Materials and Methods:

Two medicinal plants garlic and thyme were used for determining Minimum inhibitory concentration against already characterized *Mycoplasma gallisepticum* isolates in Lab, according to the protocol described by Al-Momani *et al.* (2007) with minor modification. Briefly: all two plants were shade dried at room temperature and grinded to powdered form and passed through 2 mm diameter mesh. Fresh garlic and ginger 100 g of healthy and matured garlic and thyme were washed with water and cut into small pieces and liquidized using electrical blender without adding any solvent. The powdered form 100 g was soaked in 95% methanol 1000 mL from solid to solvent with a ratio of 1:10 (w/v) for 24 hours at 25 C°. Subsequently, powder of each plant was separately mixed thoroughly by keeping on shaker at 200 rpm for 2 hours and mixture was centrifuged at 4000 g for 10 minutes at 4 C°. The extracts were concentrated under vacuum at 40-50 C° using a rotary evaporator. Extracts were stored at 20 C° and were freshly dissolved in suitable solvents prior to screening for antimicrobial activity. In order to get methanol extract, supernatant of centrifuged samples was filtered through Whatman No. 4 filter paper in a beaker. Beaker was kept in rotary evaporator for methanol evaporation at 68 C° and dry crude methanol extracts of all two plants was collected for further analysis. All extracts were dissolved in dimethyl sulfoxide (DMSO) at concentration of 1100 mg/ml to prepare stock solution of each crude extract. Garlic and thyme were collected in a clean airtight bottle, and stored for antibacterial activity test (5 g / 0.5 kg of Garlic and ginger; 100% fresh). This is done according to Sutardi *et al.* (2015).

However, penicillin G (1000 IU.mL^{-1}), ampicillin (1000 µg.mL^{-1}) with thallium acetate (500 µg.mL^{-1}), included in the culture medium have been used. Mycoplasmas develop only faint or no turbidity in broth cultures; consequently alternative methods are used to measure mycoplasmal growth. These include incorporation of substrates such as glucose, arginine or urea which are either fermented or hydrolysed by the mycoplasmas causing the medium to become acid or alkaline. These pH changes are usually detected by incorporating the pH indicator phenol red, into the mycoplasma broth, a colour change denoting mycoplasmal growth. The accepted number of organisms used for tests carried out in liquid and on solid media is 10^3 to 10^5 colour changing units (ccu) per mL or colony forming units (cfu) per plate (Figure 1).

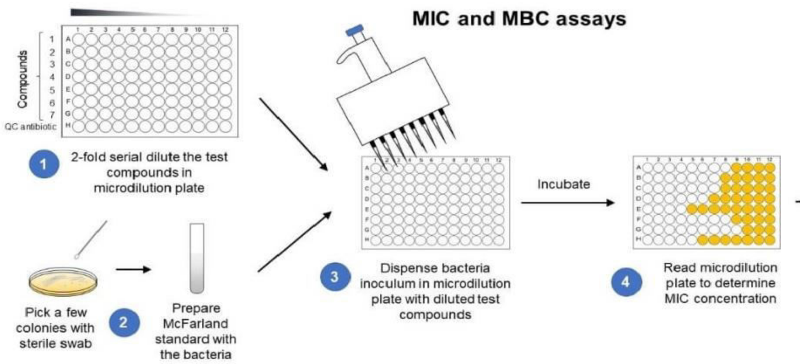

Figure 1: MIC procedure.

The minimum inhibitory concentration of the ethanol extracts that showed inhibition in the antimicrobial screening was used. The MIC was carried out by preparing the dried plant extracts in different concentrations, 125 mg / ml, 62.5 mg / ml, 31.2 mg / ml, 15.6 mg / ml, 7.8 mg / ml, 3.9 mg / ml, and 1.9 mg / ml. Extracts mixed with PPLO agar were allowed to solidify and then inoculated with mycoplasma culture. The mycoplasma plates with plant extracts were incubated for 24–48 hours at 37 C°. Minimum inhibitory concentration is defined as the lowest concentration of the extract that inhibits visible growth of the microorganism in plate. This is done according to Sutardi *et al.* (2015). Better results from extract plant and antibiotics will be used in the next experiment.

Tilmicosin	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.6 mg/ml	7.8 mg/ml	3.9 mg/ml	1.95 mg/ml	0.97 mg/ml	0.49 mg/ml
	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.6 mg/ml	7.8 mg/ml	3.9 mg/ml	1.95 mg/ml	0.97 mg/ml	0.49 mg/ml	0.24 mg/ml
	62.5 mg/ml	31.25 mg/ml	15.6 mg/ml	7.8 mg/ml	3.9 mg/ml	1.95 mg/ml	0.97 mg/ml	0.49 mg/ml	0.24 mg/ml	0.12 mg/ml
	31.25 mg/ml	15.6 mg/ml	7.8 mg/ml	3.9 mg/ml	1.95 mg/ml	0.97 mg/ml	0.49 mg/ml	0.24 mg/ml	0.12 mg/ml	0.06 mg/ml
Enrofloxacin	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.12 mg/ml	1.56 mg/ml	0.78 mg/ml	0.39 mg/ml	0.19 mg/ml
	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.12 mg/ml	1.56 mg/ml	0.78 mg/ml	0.39 mg/ml	0.19 mg/ml	0.09 mg/ml
	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.12 mg/ml	1.56 mg/ml	0.78 mg/ml	0.39 mg/ml	0.19 mg/ml	0.09 mg/ml	0.04 mg/ml
	12.5 mg/ml	6.25 mg/ml	3.12 mg/ml	1.56 mg/ml	0.78 mg/ml	0.39 mg/ml	0.19 mg/ml	0.09 mg/ml	0.04 mg/ml	0.02 mg/ml

Thyme	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.6 mg/ml	7.8 mg/ml	3.9 mg/ml	1.95 mg/ml	0.97 mg/ml
	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.6 mg/ml	7.8 mg/ml	3.9 mg/ml	1.95 mg/ml	0.97 mg/ml	0.48 mg/ml
	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.6 mg/ml	7.8 mg/ml	3.9 mg/ml	1.95 mg/ml	0.97 mg/ml	0.48 mg/ml	0.24 mg/ml
	62.5 mg/ml	31.25 mg/ml	15.6 mg/ml	7.8 mg/ml	3.9 mg/ml	1.95 mg/ml	0.97 mg/ml	0.48 mg/ml	0.24 mg/ml	0.12 mg/ml
Garli	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.6 mg/ml	7.8 mg/ml	3.9 mg/ml	1.95 mg/ml	0.97 mg/ml

	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.6 mg/ml	7.8 mg/ml	3.9 mg/ml	1.95 mg/ml	0.97 mg/ml	0.48 mg/ml
	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.6 mg/ml	7.8 mg/ml	3.9 mg/ml	1.95 mg/ml	0.97 mg/ml	0.48 mg/ml	0.24 mg/ml
	62.5 mg/ml	31.25 mg/ml	15.6 mg/ml	7.8 mg/ml	3.9 mg/ml	1.95 mg/ml	0.97 mg/ml	0.48 mg/ml	0.24 mg/ml	0.12 mg/ml

Results:

The in vitro activities of tilmicosin, enrofloxacin, garlic and thyme against the isolates of *Mycoplasma gallisepticum*, as determined by the micro-broth technique, are shown in (Table 1, 2, 3 and 4). Of the two antimicrobials and two plant extracts, enrofloxacin had the highest more than tilmicosin, while garlic had the highest plant extract than the thyme extract MIC values. Against the four used drugs as anti-mycoplasma isolates of *Mycoplasma gallisepticum* by the micro-broth method Tilmicosin had MICs of 7.8 mg/ml for the *Mycoplasma gallisepticum* (Table 1). Enrofloxacin showed the best in vitro activity giving MICs of 1.56 mg/ml for *Mycoplasma gallisepticum* (Table 2). Also, garlic had MICs of 0.97 mg/ml for *Mycoplasma gallisepticum* (Table 3). Thyme had MICs of 15.6 mg/ml for the *Mycoplasma gallisepticum* (Table 4). According to the results all *Mycoplasma gallisepticum* strains are sensitive to tilmicosin, enrofloxacin, garlic and thyme.

Table 1: *Mycoplasma gallisepticum* inoculums in microdilution plate with tilmicosin antibiotics.

250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.6 mg/ml	7.8* mg/ml	3.9 mg/ml	1.95 mg/ml	0.97 mg/ml	0.49 mg/ml
125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.6 mg/ml	7.8* mg/ml	3.9 mg/ml	1.95 mg/ml	0.97 mg/ml	0.49 mg/ml	0.24 mg/ml
62.5 mg/ml	31.25 mg/ml	15.6 mg/ml	7.8* mg/ml	3.9 mg/ml	1.95 mg/ml	0.97 mg/ml	0.49 mg/ml	0.24 mg/ml	0.12 mg/ml
31.25 mg/ml	15.6 mg/ml	7.8* mg/ml	3.9 mg/ml	1.95 mg/ml	0.97 mg/ml	0.49 mg/ml	0.24 mg/ml	0.12 mg/ml	0.06 mg/ml

Table 2: *Mycoplasma gallisepticum* inoculums in microdilution plate with enrofloxacin antibiotics.

100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.12 mg/ml	1.56* mg/ml	0.78 mg/ml	0.39 mg/ml	0.19 mg/ml
50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.12 mg/ml	1.56* mg/ml	0.78 mg/ml	0.39 mg/ml	0.19 mg/ml	0.09 mg/ml
25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.12 mg/ml	1.56* mg/ml	0.78 mg/ml	0.39 mg/ml	0.19 mg/ml	0.09 mg/ml	0.04 mg/ml
12.5 mg/ml	6.25 mg/ml	3.12 mg/ml	1.56* mg/ml	0.78 mg/ml	0.39 mg/ml	0.19 mg/ml	0.09 mg/ml	0.04 mg/ml	0.02 mg/ml

Table 3: *Mycoplasma gallisepticum* inoculums in microdilution plate with garlic plant extract.

500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.6 mg/ml	7.8 mg/ml	3.9 mg/ml	1.95 mg/ml	0.97* mg/ml
250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.6 mg/ml	7.8 mg/ml	3.9 mg/ml	1.95 mg/ml	0.97* mg/ml	0.48 mg/ml
125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.6 mg/ml	7.8 mg/ml	3.9 mg/ml	1.95 mg/ml	0.97* mg/ml	0.48 mg/ml	0.24 mg/ml
62.5 mg/ml	31.25 mg/ml	15.6 mg/ml	7.8 mg/ml	3.9 mg/ml	1.95 mg/ml	0.97* mg/ml	0.48 mg/ml	0.24 mg/ml	0.12 mg/ml

Table 4: *Mycoplasma gallisepticum* inoculums in microdilution plate with thyme plant extract.

500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.6* mg/ml	7.8 mg/ml	3.9 mg/ml	1.95 mg/ml	0.97 mg/ml
250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.6* mg/ml	7.8 mg/ml	3.9 mg/ml	1.95 mg/ml	0.97 mg/ml	0.48 mg/ml
125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.6* mg/ml	7.8 mg/ml	3.9 mg/ml	1.95 mg/ml	0.97 mg/ml	0.48 mg/ml	0.24 mg/ml
62.5 mg/ml	31.25 mg/ml	15.6* mg/ml	7.8 mg/ml	3.9 mg/ml	1.95 mg/ml	0.97 mg/ml	0.48 mg/ml	0.24 mg/ml	0.12 mg/ml

Discussion:

Natural products of animals, plants and microbial sources have been used by man for thousands of years either in the pure forms or crude extracts to treat many diseases

(Parekh and Chanda, 2007). Garlic (*Allium sativum L.*) is one of those plants that were seriously investigated over several years and used for centuries to fight infectious diseases (Onyeagba *et al.*, 2004). The taxonomic position of garlic and related genera had been a matter of controversy for long period of time. The most recent classification scheme of garlic was class Liliopsida, subclass Liliidae, superorder Liliianae, order Amary-llidales, family Alliaceae, subfamily Allioideae, tribe Allieae and genus Allium which is mainly based on the sequences of nuclear ribosomal DNA (Friesen *et al.*, 2006). Garlic is nicknamed as Russian penicillin for its widespread use as a topical and systemic antimicrobial agent; it is commonly used in many cultures as an excitement and reputation of healing power (Timbo *et al.*, 2006). Garlic contains at least 33 sulfur compounds, several enzymes and the minerals germanium, calcium, copper, iron, potassium, magnesium, selenium and zinc; vitamins A, B1 and C, fiber and water. It also contains 17 amino acids to be found in garlic: lysine, histidine, arginine, aspartic acid threonine, swine, glutamine, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tryptophan and phenylalanine (Josling, 2005). Although allicin is considered the major antioxidant and scavenging compound, recent studies showing that other compounds may play stronger roles; such as polar compounds of phenolic and steroidal origin, which offer various pharmacological properties without odor and are also heat stable (Lanzotti, 2006). many researches had demonstrated its effectiveness and broad spectrum antimicrobial activity against many species of bacteria, viruses, parasites, protozoan and fungi (Jaber and Al-Mossawi, 2007). Garlic is more effective with least side effects as compared to commercial antibiotics; as a result, they are used as an alternative remedy for treatment of various infections (Tepe *et al.*, 2004). Out of the many medicinal plants, garlic has an antimicrobial property which protects the host from other pathogens highlighting the importance of search for natural antimicrobial drugs (Bajpai *et al.*, 2005; Wojdylo *et al.*, 2007). Previously conducted researches confirmed that garlic is not only effective against Gram positive and Gram negative bacteria but also possess antiviral and antifungal activities (Tsao and Yin, 2001).

Thyme is cultivated in most of the European countries, together with France, Svizzera, Spain, Italy, Bulgaria, Portuguese Republic and Ellas. Yield and quality of oil varies in line with the genetic make-up of stuff, crop maturity at harvest, setting and distillation follow (Reddy *et al.*, 2014). Thyme has been thought of to be antiseptic, antimicrobial, medication, astringent, anthelmintic, medicinal drug, carminative, disinfectant, medicinal drug and tonic. Thyme is incredibly useful in cases of assorted intestinal infections and infestations, like hookworms, ascarids, gram-positive and gram-negative bacterium, fungi and yeasts as well as Candida albicans. Its active constituent, thymol, is active against enterobacteria and cocci bacteria (Reddy *et al.*, 2014). Thyme may also improve liver functioning, and act as an appetite stimulant. It will be used in treatment of cartilaginous tube, bronchial and urinary infections. Used as a gargle, Thyme is helpful in treatment of laryngitis and inflammation. The main component of the volatile oil of thyme, thymol, is active against enterobacteria and cocci bacteria. It is used for skin issues like oily skin, sciatica, acne, dermatitis, skin condition and bug bites. In aromatherapy, the distinct types, thymol, 'red thyme oil', linalol kind for its terribly light soft action and thuyanol for antiviral properties are used. A corrected product, 'white thyme oil' is also used, and it's milder on the skin (Reddy *et al.*, 2014). Applied to the skin, thyme relieves bites and stings, and relieves neuralgy and rheumatic aches and pains. The essential oils obtained from *Thymus vulgaris L.* harvested at 4 biological process stages were evaluated for their biological activity and chemical components. The thyme volatile oils were analysed for their inhibition effects against 9 strains of gram-negative bacteria and 6 strains of gram-positive bacteria. The bioimpedance methodology was chosen for finding out the antibacterial activity of the essential oils and also the parameter chosen for outlining and quantifying the antibacterial activity of the thyme oils was the detection time (Reddy *et al.*, 2014). The plate counting technique was used to studying the inhibitory effect by direct exposure. All the thyme essential oils examined had a significant bacteriostatic activity against the microorganisms tested. This activity was additional marked against the gram-positive bacteria. The oil from thyme fully flower was the foremost effective at stopping the growth of the microorganism species examined. The oils tested were conjointly shown to possess smart antibacterial activity by direct contact, that gave the impression to be a lot of marked against the gram-negative microorganism (Reddy *et al.*, 2014). The essential oil from *T. vulgaris* showed a high content of oxygenated monoterpenes 56.53% and low contents of monoterpene hydrocarbons 28.69%, sesquiterpene hydrocarbons 5.04% and oxygenated sesquiterpenes 1.84% (Al-Maqtari *et al.*, 2011). The predominant compound among the essential oil components was thymol 51.34% while the amount of all other components of the oil was less than 19%.

Enrofloxacin or 1-Cyclopropyl-6-fluoro-7-(4-ethyl-1-piperazinyl)-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid, belongs to fluoroquinolone family which is a subfamily of quinolone. The first quinolone is the Nalidixic acid used in animal at the beginning of 1980s, enrofloxacin is the first fluoroquinolone patented in 1984 (Grohe *et al.*, 1987). The huge evolution in the quinolone family is the addition of a fluor atom on the 6th position which improves quinolones' antibacterial spectrum (Wright *et al.*, 2000) and creates the fluoroquinolone subfamily. The marketing authorization reports a large antimicrobial spectrum for enrofloxacin, which is efficient on most gram-negative and gram-positive bacteria but not efficient on anaerobic bacteria (Fauchier, 2013). Enrofloxacin is a powerful antimicrobial which have shown efficacy against a lot of bacterial diseases (CVMP, 2007; Sarkozy, 2001). The effectiveness of enrofloxacin against some bacterial infections in cattle, poultry, domestic carnivores (dogs and cats), rodents, lagomorphs and crustaceans has been assessed in many published studies, as well during natural infections as during experimental infections. Among these studies, some relate to classical infections contained in the Summary of Product Characteristics (SPC) of veterinary products but some evaluate the efficacy of enrofloxacin in species for which there is no marketing authorisation or against specific bacteria such as *Anaplasma marginale* in cattle, *Ehrlichia canis* and *Brucella canis* in dogs, *Bartonella henselae* or *Bartonella clarridgeiae* and *Chlamydophila felis* in cats, *Toxoplasma gondii* in *Calomys callosus* or even Vibrio harveyi in Artemia franciscana (Trouchon and Lefebvre, 2016).

Tilmicosin is a semisynthetic, broad-spectrum, bacteriostatic macrolide antibiotic synthesized from tylosin for veterinary uses (Kempf *et al.*, 1997). Tilmicosin offers promising prospect of application in clinical veterinary practices. It is a useful drug for treatment and control of respiratory diseases due to its high volume of distribution, long half-life and preferable accumulation in lung. Tilmicosin is used for control and treatment of respiratory diseases caused by Mycoplasma spp (Abu-Basha *et al.*, 2007). Chicken must not be slaughtered before 4 days from the stopping of tilmicosin administration (Mossad Elsayed *et. al*, 2014). Macrolide antibiotics are structurally similar drugs with bacteriostatic effect. Large number of drugs in this class had been isolated from genus Streptomyces of the soil bacteria (Papich *et al.*, 2001). Antibiotics besides their antibacterial effects its will be better is also help the wound healing process so it would be beneficial to clearly identify if tilmicosin ointment help the healing process. Adverse or beneficial events associated with drug users have an important impact especially in commonly used drugs for well use of drugs in multiple forms, dispensing or administrations (Gama, 2008). Tilmicosin commonly used idrug in veterinary medicine and at high dose has some adverse effects such as anaphylaxis, cardiotoxic, collapse and transient swelling at the site of injection (Gheith *et al.*, 2015) but also has beneficial activities must be explored. To the best of our knowledge no previous studies reported about effects of tilmicosin on wound healing, ulcer treatment, hypnotic and relaxant effects on intestine or on utras (Abo El-Ela and El-Banna, 2017).

Conclusion:

According to the results all *Mycoplasma gallisepticum* strains are sensitive to all antibiotics and plants extracts in different level and the highest effectiveness was for the garlic.

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