

## EFFICACY OF QUINOLONES AND AMINOGLYCOSIDES AGAINST BOVINE AND OVINE MYCOPLASMA

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### Abstract

Lung samples with peumonic lesions and lymph nodes were collected from cattle and sheep and examined for mycoplasma. All the isolates recovered from cattle were identified as *Mycoplasma bovirhinis* (14.13%). *Mycoplasma ovipneumoniae* could be detected in 12.9% of sheep lungs, while, 4 isolates (6.45%) from lung samples and 7 (11.29%) from lymph nodes were identified as *M.arginini*.

The efficacy of some antimicrobial agents were tested in vitro against the isolates by the metabolic inhibition test. Enrofloxacin had the highest minimal inhibitory concentration (M.I.C.) values (0.003-0.048 µg/ml). *Mycoplasma bovirhinis* and *M.arginini* were sensitive to pefloxacin, erythromycin and lincospectin (0.024-0.19 µg/ml), while *M.ovipneumoniae* was less susceptible (0.097-0.39 µg/ml).

Ampicillin and streptomycin had the lowest M.I.C. values for all the examined isolates (0.78-3.12 µg/ml).

### INTRODUCTION

Kumar *et al.* (1989) studied the *in-vitro* antibiotic sensitivity of mycoplasma and acholplasma isolates recovered from genital and respiratory tracts of cattle and buffaloes. The isolates were sensitive to streptomycin, and resistant to neomycin. Bradbury *et al.* (1994) found that *Mycoplasma gallisepticum* and *M.synoviae* were sensitive to danofloxacin with M.I.Cs ranging from 0.008 to 0.5 µg/ml. Abd El-Rahman (1995) tested the sesitivity of *M.bovirhinis* isolated from feeder calves to some antibiotics. Lincospectin had the highest activity with M.I.C. of 0.5-2 µg/ml, while, streptomycin had M.I.C. of 4-10 µg/ml. Eissa (1996) recorded that enrofloxacin and ciprfloxacin had strong inhibitory effect on some avian mycoplasmas. The M.I.Cs. were 0.006-0.048 µg/ml, while, streptomycin had less effect on most tested strains (0.39-1.56 µg/ml).

The purpose of the present study was to compare and evaluate the efficacy of quinolones and aminoglycosides against bovine and ovine mycoplasma.

## MATERIALS AND METHODS

### Samples

One hundred and twenty-four samples were collected from sheep (62 lungs and 62 lymph nodes), and 92 samples from cattle (46 lungs and 46 lymph nodes). The lungs showed pneumonic lesions and they were obtained from Cairo abattoir.

### Media

Media used for the propagation of mycoplasma and biochemical characterization were prepared as described by Erno and Stipkovits (1973). The isolates were serologically identified by growth inhibition test (Clyde, 1964).

### Antimicrobials

Name of antimicrobial drugs and the form in which they were available and source are listed in Table 1.

### Sterile stock solutions

Sterile stock solutions containing 1000 µg/ml were prepared from each drug in distilled water. They were stored at 4°C and used freshly.

### Titration of mycoplasmas

For each mycoplasma isolate, the number of colour changing units (CCU) was determined by the method described by Taylor Robinson (1983). A titer of  $10^3$ - $10^4$  CCU/0.2 ml was required for the test proper (Senterfit, 1983).

### Determination of minimal inhibitory concentration (M.I.C.)

The test was performed in duplicate exactly as described by Senterfit (1983). The antimicrobials were tested in serial twofold dilutions at concentrations ranging from 12.5 to 0.003 µg/ml.

### Endpoint readings

The M.I.C. was the lowest concentration of the antimicrobial that completely prevented a colour change. This typically occurred after 1 to 2 days. For

comparison, a final reading was taken after 14 days incubation (Whithear *et al.*, 1983). Results were expressed in  $\mu\text{g/ml}$  of active compound.

Table 1. Antimicrobial drugs used for M.I.C. determination.

Drug	Group	Form used	Source
Enrofloxacin	Quinolones	Solution (10%)	Amoun Pharmaceuticals Industries company (APIC)
Danofloxacin	Quinolones	Solution (10%)	Pfizer, Egypt.
Pefloxacin	Quinolones	Powder (5%)	Hoechst-Roussel Pharmaceuticals
Gentamycin	Aminoglycosides	Solution (10%)	Alex. Company for Chemicals and Pharmaceuticals.
Lincospectin	Aminoglycosides	Solution (10%)	Egypt. Company for Chemicals and Pharmaceuticals
Streptomycin	Aminoglycosides	Powder (720mg/gm)	(ADWIA).
Erythromycin	Macrolide	Powder (20%)	ADWIA
Ampicillin	Modified Penicillin	Powder (20%)	Bremer Pharmaceuticals, GMBA, Germany

## RESULTS

The results in Table 2 showed that, 10 out of 46 examined lung samples from cattle were positive (21.74%), while, the recovery rate of mycoplasma from sheep lungs was 19.35%. The isolation of mycoplasma from lymph nodes of sheep was higher than that from cattle (11.29% and 6.52, respectively). Genus determination revealed that, all the isolates belonged to genus mycoplasma. The serological identification of cattle isolates showed that they all belonged to *Mycoplasma bovirhinis*. Concerning the isolates from sheep lungs, 8 (12.9%) isolates were identified as *M.ovipneumoniae* and 4 (6.45%) *M.arginini*. All the isolates recovered from sheep lymph nodes were identified as *M.arginini* (11.29). The *in-vitro* activities of antibiotics against mycoplasma isolates recovered from cattle and sheep as determined by metabolic inhibition technique, are shown in Table 3. Enrofloxacin had the highest M.I.C. values for all the tested isolates (0.003 - 0.048  $\mu\text{g/ml}$ ). *Mycoplasma bovirhinis* and *M.arginini* were sensitive to pefloxacin, erythromycin and lincospectin (0.024 - 0.19  $\mu\text{g/ml}$ ), while, *M.ovipneumoniae* was less susceptible (0.097 - 0.39  $\mu\text{g/ml}$ ). In general, ampicillin and streptomycin had the lowest M.I.C. values for all the examined isolates (0.78-3.12  $\mu\text{g/ml}$ ).

Table 2. Primary isolation and serological identification of mycoplasma recovered from cattle and sheep.

Animal	Organ	Number of samples examined	Digtonin	Biochemical pattern			Number of positive samples	Percentage of positive samples %	Serological identification
				G	A	F&S			
Cattle	Lung Lymph node	46	+	-	+	10	21.74	<i>M. bovirhinis</i> <i>M. bovirhinis</i>	
		46	+	-	+	3	6.52		
Sheep	Lung Lymph node	62	+	-	-	8	12.9	<i>M. ovipneumoniae</i> <i>M. arginini</i> <i>M. arginini</i>	
		62	+	+	-	4	6.45		
			-	+	-	7	11.29		

G = Glucose

A = Arginine

F &amp; S = Film and spot

\* = Late reaction

Table 3. In vitro activities of seven antimicrobials against mycoplasma isolates recovered from cattle and sheep.

Mycoplasma isolates	Minimal Inhibitory Concentrations (M.I.Cs)							
	Enrofloxacin	Danofloxacin	Pefloxacin	Gentamycin	Lincospectin	Erythromycin	Ampicillin	Streptomycin
<i>M.ovipneumoniae</i>	0.024-0.048	0.097-0.19	0.39-0.78	0.048-0.19	0.19-0.78	0.097-0.39	0.78-1.56	1.56-3.12
<i>M.bovirhinis</i>	0.003-0.12	0.006-0.048	0.024-0.097	0.006-0.024	0.024-0.19	0.024-0.048	0.78-1.56	0.78-3.12
<i>M.arginini</i>	0.003-0.006	0.012-0.048	0.024-0.048	0.012-0.048	0.024-0.097	0.048-0.097	0.78-1.56	0.78-1.56

M.I.C. ( $\mu\text{g/ml}$ )

Colour Changing Units (CCU) of mycoplasma isolates  $10^3$ - $10^4$ /0.2 ml.

Interpretation of results was according to Bradbury et al. (1994).

Sensitive : 0.006-0.05  $\mu\text{g/ml}$ .

Intermediate : 0.4-2.0  $\mu\text{g/ml}$ .

Resistant : > 3.0  $\mu\text{g/ml}$ .

## DISCUSSION

*Mycoplasma ovipneumoniae* is the most commonly isolated mycoplasma from the ovine respiratory tract (Sullivan *et al.*, 1973, Jones *et al.*, 1979, Cottew and Yeats, 1981), though implicated in the aetiology of ovine atypical pneumonia (Foggie *et al.*, 1976, Alley and Charke, 1979, Jones *et al.*, 1982).

In the present study, *M.ovipneumoniae* could be detected in 12.9% of the examined sheep lungs with pneumonic lesions. *Mycoplasma bovirhinis* was isolated from lungs and lymph nodes of cattle (21.74% and 6.52%, respectively). Our results are in agreement with Knudtson *et al.*, (1986) and Abd El-Rahman (1995) who isolated *M.bovirhinis* from calves with clinical pneumonia. *Mycoplasma arginini* was detected in 6.45% of lungs and 11.29% of lymph nodes of sheep. *Mycoplasma arginini* was first isolated from sheep and goats by Barile *et al.* (1968).

Antibiotic sensitivity testing of mycoplasma can be carried out on agar medium using similar methods to those for bacteria, but the broth method is preferred because of the correlation between M.I.C. and inhibitory zone for inhibitory zone for most mycoplasma - antimicrobial combinations (Senterfit, 1983).

Our results proved that, enrofloxacin had the highest activity against all tested isolates (0.003 - 0.048  $\mu\text{g/ml}$ ), followed by danofloxacin and gentamycin (0.006 - 0.19  $\mu\text{g/ml}$ ). These results are in agreement with Bradbury *et al.* (1994) who recorded that *M.gallisepticum* was susceptible to danofloxacin (0.008 - 0.25  $\mu\text{g/ml}$ ), and Eissa (1996) who found that the all tested mycoplasma strains (*M.gallisepticum*, *M.synoviae*, *M.pullorum* and *M.iowae*) were sensitive to enrofloxacin (0.006 - 0.048  $\mu\text{g/ml}$ ).

In the present study, *M.bovirhinis* and *M.arginini* were sensitive to pefloxacin, erythromycin and lincospectin (0.024-19  $\mu\text{g/ml}$ ), while, *M.ovipneumoniae* less sensitive (0.097-0.39  $\mu\text{g/ml}$ ). Abd El-Rahman (1995) found that *M.bovirhinis* was sensitive to lincospectin.

Our results revealed that, ampicillin and streptomycin had the lowest activities against all tested isolates (0.78 - 3.12  $\mu\text{g/ml}$ ). Eissa (1996) recorded that streptomycin had less effect on most tested mycoplasma strains (*M.gallisepticum*, *M.synoviae*, *M.pullorum* and *M.iowae*) (0.39-1.56  $\mu\text{g/ml}$ ).

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## فعالية الكينولونز والأمينوجليكوسايدز ضد ميكوبلازما الأبقار والأغنام

صبرى عيسى ، سعيد الشاطر ، منسى دردير

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تم تجميع عدد ١٠٨ عينه من الرئة المصابة بالتهاب رئوى وكذلك من الغدد الليمفاوية من الأبقار والأغنام من مجزر القاهرة وتم فحصها للميكوبلازما. وجد أن جميع العينات المعزولة من الأبقار تنتمي إلى الميكوبلازما بوفيرنيس (١٣ ، ١٤)، بينما تم عزل الميكوبلازما أو فينيمونى بنسبة ١٢,٩٪ من الأغنام، فى حين أمكن عزل الميكوبلازما أرجينى بنسبة ٦,٤٥٪، ١١,٢٩٪ على التوالي.

تم إجراء إختيار مانع الميتابوليزم لتحديد فعالية بعض المضادات الحيوية الحديثة ضد العتقات المعزولة. وقد تبين أن الإنروفلوكساسين هو أكثر فعالية ضد جميع المعزولات. ووجد أن الميكوبلازما بوفيرنيس والميكوبلازما أرجينى حساسة للبيفلوكساسين، إريثروميسين واللينكوسبكتين، بينما الميكوبلازما أوفينيمونى كانت أقل حساسية.

تبين أن الأمبسلين والإستربتوميسين لهما أقل تأثير على جميع المعزولات.