EFFICACY OF QUINOLONES AND AMINOGLYCOSIDES AGAINST BOVINE AND OVINE MYCOPLASMA

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Abstract

Lung samples with pneumonic lesions and lymph nodes were collected from cattle and sheep and examined for mycoplasma. All the isolates recovered from cattle were identified as Mycoplasma bovis (14.13%). Mycoplasma ovipneumoniae could be detected in 12.9% of sheep lungs, while, 4 isolates (5.45%) 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 bovis and M. arginini were sensitive to pefloxacin, erythromycin and lincomycin (0.024-0.19 μg/ml), while Mycoplasma 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 acholeplasma 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. bovis isolated from feeder calves to some antibiotics. Lincopectin 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 ciprofloxacin 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 $10^2$-$10^4$ 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 μg/ml of active compound.

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

<table>
<thead>
<tr>
<th>Drug</th>
<th>Group</th>
<th>Form used</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrofloxacin</td>
<td>Quinolones</td>
<td>Solution (10%)</td>
<td>Amoun Pharmaceutics Industries company (APC)</td>
</tr>
<tr>
<td>Danofloxacin</td>
<td>Quinolones</td>
<td>Solution (10%)</td>
<td>Pfizer, Egypt.</td>
</tr>
<tr>
<td>Pefloxacin</td>
<td>Quinolones</td>
<td>Powder (5%)</td>
<td>Hoechst-Roussel Pharmaceuticals</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>Aminoglycosides</td>
<td>Solution (10%)</td>
<td>Alex. Company for Chemicals and Pharmaceuticals</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>Aminoglycosides</td>
<td>Solution (10%)</td>
<td>Egypt. Company for Chemicals and Pharmaceuticals</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>Aminoglycosides</td>
<td>Powder (720mg/gm)</td>
<td>(ADWIA).</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>Macrolide</td>
<td>Powder (2%)</td>
<td>(ADWIA)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>Modified Penicillin</td>
<td>Powder (20%)</td>
<td>Bremer Pharmaceutics, GMBA, Germany</td>
</tr>
</tbody>
</table>

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 bovis. Concerning the isolates from sheep lungs, 8 (12.9%) isolates were identified as M.ovicapniae and 4 (6.5%) 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 μg/ml). Mycoplasma bovis and M.arginini were sensitive to pefloxacin, erythromycin and lincomycin (0.024 - 0.19 μg/ml), while, M.ovicapniae was less susceptible (0.097 - 0.39 μg/ml). In general, ampicillin and streptomycin had the lowest M.I.C. values for all the examined isolates (0.78-3.12 μg/ml).
Table 2. Primary isolation and serological identification of mycoplasma recovered from cattle and sheep.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Organ</th>
<th>Number of samples examined</th>
<th>Digiton</th>
<th>G</th>
<th>A</th>
<th>F&amp;S</th>
<th>Number of positive samples</th>
<th>Percentage of positive samples %</th>
<th>Serological identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>Lung</td>
<td>46</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>*</td>
<td>10</td>
<td>21.74</td>
<td>M. bovirhinis</td>
</tr>
<tr>
<td></td>
<td>Lymph node</td>
<td>46</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>6.52</td>
<td>M. bovirhinis</td>
</tr>
<tr>
<td>Sheep</td>
<td>Lung</td>
<td>62</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>8</td>
<td>12.9</td>
<td>M. ovipneumoniae</td>
</tr>
<tr>
<td></td>
<td>Lymph node</td>
<td>62</td>
<td></td>
<td></td>
<td>+</td>
<td>-</td>
<td>4</td>
<td>6.45</td>
<td>M. arginini</td>
</tr>
</tbody>
</table>

G = Glucose  
A = Arginine  
F & S = Film and spot  
* = Late reaction
Table 3. In vitro activities of seven antimicrobials against mycoplasma isolates recovered from cattle and sheep.

<table>
<thead>
<tr>
<th>Mycoplasma Isolates</th>
<th>Minimal Inhibitory Concentrations (M.I.Cs)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enrofloxacin</td>
<td>Danofoxacin</td>
</tr>
<tr>
<td><strong>M. ovipneumoniae</strong></td>
<td>0.024-0.048</td>
<td>0.097-0.19</td>
</tr>
<tr>
<td><strong>M. bovirhinis</strong></td>
<td>0.003-0.012</td>
<td>0.006-0.048</td>
</tr>
<tr>
<td><strong>M. arginini</strong></td>
<td>0.003-0.006</td>
<td>0.012-0.048</td>
</tr>
</tbody>
</table>

M.I.C. (µg/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 µg/ml.
Intermediate: 0.4-2.0 µg/ml.
Resistant: > 3.0 µg/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 bovis*/*hinii* 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. bovis*/*hinii* from calves with clinical pneumonia. *Mycoplasma argini* was detected in 6.45% of lungs and 11.29% of lymph nodes of sheep. *Mycoplasma argini* 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, enrofloxacain had the highest activity against all tested isolates (0.003 - 0.048 μg/ml), followed by danofroxicain and gentamycin (0.006 - 0.19 μg/ml). These results are in agreement with Bradbury et al. (1994) who recorded that *M. gallisepticum* was susceptible to danofloxacain (0.008 - 0.25 μg/ml), and Eissa (1996) who found that the all tested mycoplasma strains (*M. gallisepticum, M. synoviae, M. pullorum* and *M. iowae*) were sensitive to enrofloxacain (0.006 - 0.048 μg/ml).

In the present study, *M. bovis*/*hinii* and *M. argini* were sensitive to IVoxacin, erythromycin and linospectin (0.024-19 μg/ml), while, *M. ovipneumoniae* less sensitive (0.097-0.39 μg/ml). Abd El-Rahman (1995) found that *M. bovis*/*hinii* was sensitive to linospectin.

Our results revealed that, ampicillin and streptomycin had the lowest activities against all tested isolates (0.78 - 3.12 μg/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 μg/ml).
REFERENCES


فعالية الكينولونز والأمينوجليكوسباسيدز ضد ميکروبلازما الأبقار والأفغام

صبري عربى - معهد بحوث صحة الحيوان - مركز البحوث الزراعية - الدقى - جبزة

معهد بحوث صحة الحيوان - مركز البحوث الزراعية - الدقى - جبزة

تتم تجميع عدد 18 عينة من الرئة المصابه بالالتهاب المزمن وكذلك من الغدد الليمفاوية من الأبقار والأفغام من مزرعة القاهرة وتتم فحصها لميکروبلازما. وجد أن جميع العينات الم محللة من الأبقار تشتت إلى الميکروبلازما بوڤيروفينيس (12.4%) بينما تم عزل الميکروبلازما أو فينيميسوسون بنسبة 12.4% من الأفغام، في حين اكتشاف عزل الميکروبلازما أرجينيني بنسبة 4.4%, 2.8%, 1.1% على التوالي.

تم إجراء اختبارات الحساسية لتحديد فعالية بعض المضادات الحيوية المклиفة ضد العوامل الم محللة. وقد تبين أن الإفروفلكساسين هو أكثر فعالية ضد جميع العوامل. ووجد أن الميکروبلازما بوڤيروفينيس والميکروبلازما أرجينيني حساسة للفلافوكساسين، إيجيروفينيس والفينيميسوسون، بينما الميکروبلازما أرجينيني كانت أقل حساسية.

تبيّن أن الأمبيسيلين والإستريوتوميسين لهما أقل تأثير على جميع العوامل.