قسم: أمراض الدواجن
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رئيس القسم: د. ابراهيم حسن سكر

اختبار حساسية عوشرات الميكولا زما والبكتيريا المصاحبة لها
والتحسين زلة من الدجاج والرمي والبط. بصعيد مصر العليا

ابراهيم سكر، عادل سليمان، صلاح موسى، محسن الدمرداش

تم تجميع مسحات من الحنجرة والحيوب الأنفية والاكسيا الهوائي المعدن
الدجاج والرمي والبط وقد ثبت وجود ميكولا زما جاليستمب بنسبية 24 - 26 %
في الدجاج والرمي بالترتيب وم. ملبجريس في الرمي بنسبة 20 % بينما تتم
عزل م.ألانينس (14 %)، أ. ليد لا ود (28 %)، أ.اكزيم (32 %) من البط
وكانت هذه العوشرات مصاحبة للميكروب القولوني، ميكروب السيد، ماناس،
والميكروب العنقودي الذهبي. وميكروب السالمونيلا، بللوم. وقد أوضح البحث
أن هذه العوشرات عالية الحساسية للجرعات السيسين ومتوسطة الحساسية
لللينكوبميسين وغير حساسة لبقية مضادات الحيوي المستخدمة.

ويعتبر ام طريقة تغطيس ببعض الرومي المصاب بالميكولا زما في تركيزات
مختلفة من مضادات الحيوي فقد ثبت أن استقرار الجواميسين تسبب في
نسبة وضعية وجود الميكروب وثلاج الدوكسي سيكلين ثم جاء بعد النايلاين.
IN-VITRO SENSITIVITY OF MYCOPLASMAS AND ASSOCIATED BACTERIA
ISOLATED FROM CHICKENS & TURKEYS AND DUCKS
AT THE AREA OF UPPER EGYPT
(With 3 Tables)

By
L.M. SOKKAR; A.M. SOLIMAN; S. MOUSA and M.Z. EL-DEMERDASH
(Received at 21/3/1985)

SUMMARY
Swabs from trachea, sinuses and air-sacs taken from chickens, turkeys and ducks of different ages revealed the presence of M. gallisepticum (24 and 32%) in chickens and turkeys respectively, M. meleagridis (30%) were detected in infected turkeys, while M. anatis (16%), M. gallinarum (24%), M. iners (14%), A. laidlawi (28%) and A. aphanum (32%) were recovered from ducks. These isolates were found in association with E. coli, Pseud. aeruginosa, Staph. aureus and S. gallinarum pullorum. All isolates showed high sensitivity to josamycin, moderate sensitivity to lincomycin and low variable sensitivity to other antibiotics. Incubated turkey-eggs from infected farm showed good elimination of mycoplasma after diploing in josamycin solution, while inferior results were obtained with doxycycline and tylosin-tartrate treatment.

INTRODUCTION
Mycoplasmas have been isolated from man, animals and birds, most of these mycoplasmas are pathogenic and cause specific diseases. Mycoplasmas were first isolated from poultry (NELSON, 1935). Several disease conditions were reported due to mycoplasma species. CRD in chickens and sinusitis in turkeys caused by M. gallisepticum was described by AMIRA (1976) and SOLIMAN (1982). Air-saccultitis in turkey poult, late incubation mortality and poor growth rate due to M. meleagridis were reported by GHAZIKHANIAN and YAMAMOTO (1974). Air-saccultitis and ascitis in ducks due to M. anatis, M. gallinarum, M. iners, A. laidlawi and A. aphanum were described by ROBERTS (1964), KARPAKAS (1969), EL-EBEEDY (1976), FAZIA (1976) and SOLIMAN, (1985). Complicated air-saccultitis in turkey- poult infected with M. meleagridis and E. coli were recorded by MOHAMED et al. (1970). Also E. coli, Staph. aureus, Pseud. aeruginosa and S. gallinerum pullorum were recovered from upper and lower respiratory organs by BERGMAN, et al. (1980) and KIBENG and WILCOX, (1983). MURATA, et al. (1981) and SOLIMAN, (1982, 1985) tested the effect of several antibiotics on mycoplasma isolates, and reported greater drug resistance to tylosin-tartrate. Also RAO, et al. (1976) showed that 98.8, 93.3, 46.6 and 76% of 345 E. coli strains were resistant to erythromycin, streptomycin, oxytetracycline and chlorotetracycline respectively. YOON, et al. (1981) and EL-BAKRY (1983) applied the in-vitro sensitivity test on E. coli, S. gallinarum pullorum Staph. aureus and Pseud. aeruginosa, they reported the greatest resistance of various E. coli strains and Pseud. aeruginosa to erythromycin, neomycin, sulphonamides and Nitrofurantion. An increased rate of hatchability

The present work was planned to cover the following items:
- Isolation and identification of mycoplasmas and the associated bacteria from chicken, turkey and duck flocks in the area of Upper Egypt.
- Application of the in-vitro sensitivity test on the recovered strains and associated bacteria against the available drugs to choose the effective ones.
- Trials for treating infected turkey eggs by antibiotic dipping to control mycoplasma infection in turkeys.

**MATERIAL and METHODS**

I— Isolation and identification of mycoplasmas and associated bacteria

1) **Mycoplasmas**: Tracheal, sinuses and air-sac swabs were collected on Brain-Heart-infusion broth, from living and dead chickens, turkeys and ducks of different ages. Samples were cultured as described by SABRY (1968). Inoculated broth and agar media were incubated at 37°C, however agar plates were incubated in moist candle jar under reduced oxygen tension. After 3-days plates were examined microscopically for appearance of characteristic colonies. The suspected colonies were subjected to further identification: digitonine-sensitivity test (FREUNDT, et al. 1979), biochemically (SABRY, 1968 & ERNO and STIPKOVITIS, 1973) and Serologically (CLYDE, 1964 and KROGSGARD-JENSEN, 1972).

2) **Associated bacteria**: Corresponding samples to that for mycoplasmas were inoculated on nutrient-broth and incubated at 37°C. This was followed by subculturing on blood-agar, MacConky (agar, 5-5 agar and Crystal-Violet-blood agar. Subcultures were incubated at 37°C for 24 hours. Suspected colonies were picked up and subjected to further biochemical and serological identification (CRUICKSHANK, et al. 1975).

II— In-Vitro sensitivity of mycoplasma and associated bacteria against antimicrobial agents:

Sensitivity discs produced by Oxoid-laboratories, England and Ughon Company, U.S.A. were used, while sensitivity discs for josamycin (0.05 mg/ml) were prepared from "alplucine 20%" produced by Vibac-laboratories, France, and "Doxycycline" discs were prepared from "Vbavet" produced by Pfizer, New York (0.1 mg/ml). The test was carried out on mycoplasmas after CLYDE (1964), and on associated bacteria after KOLMER, et al. (1951).

III— Antibiotic treatment of naturally infected turkey-eggs with mycoplasmas

A total of 200 turkey-eggs were obtained from El-Wadi El-Gadid turkey farm near the history of mycoplasma infection. Eggs were divided into four group of 50 eggs each. Eggs were dipped in antibiotic solutions at 2-5°C for one minute. Antibiotic solutions were prepared from "alplucine, 50 p.p.m.", Vibavet 50 p.p.m." and "Tylosine-tartrate 200 p.p.m." The fourth group of eggs served as non treated control. At the end of incubation dead-embryos and hatched poults after two weeks old, were subjected to Post-mortum and mycoplasma examination.

**RESULTS**

Results of isolation and identification of mycoplasmas and associated bacteria and the in-vitro sensitivity of these isolates as well as antibiotic turkey-egg treatment are illustrated in tables 1,2,3 respectively.

IN-VITRO SENSITIVITY OF MYCO. AND ASSOCIATED BACT.

DISCUSSION

It is clear from the results of isolation and identification that mycoplasmosis in poultry constitute an important economic problem causing high losses in flocks at the area of Upper Egypt. Results of recovery of M. meleagridis from turkey air-sac of different ages agreed with FREY, et al. (1968) and YAMAMOTO (1978). As well as results of M. gallisepticum recovery from turkey sinuses exudate were agreed with SOLIMAN (1982) while its recovery from trachea, air-sacs and sinuses of chickens were agreed with AHMED (1980). The recovery of M. anatis & M. gallinarum & M. iners & A. laidlawii and A. axanthum from duck samples were parallel with observations of KARPAS and FABBRICANT (1969), FAJZIA (1976), EL-EBEEDY (1976) and SOLIMAN (1985).

Results of recovery of associated bacteria were agreed with BERGMAN, et al. (1983).

Results of the in-vitro sensitivity indicates that all mycoplasma strains and associated bacteria were highly sensitive to josamycin and varied from sensitive to weak sensitivity to other antibiotics, while drug resistance was recorded in Tylosin-tartrate, Nitrofurantoin, Neomy-cin and oxytetracycline. These results were agreed with KLEVEN, et al. (1971), ELMAHDI, et al. (1978), HAMDI, et al. (1980), YOON, et al. (1981), SOLIMAN (1982), EL-BAKRY (1983), and SOLIMAN (1985).

Results of egg-dipping in antibiotic revealed the higher sensitivity of josamycin and greater resistance to Tylosintartrate and this agreed with CHAKIKHANI and YAMAMOTO (1969), MURATA, et al. (1981).

Results of sensitivity test points to the need of periodical testing of bacterial isolates to detect their sensitivity to the available antimicrobial agents in order to choose the more effective drugs for using in the prophylactic and therapeutics programs.

REFERENCES


IN-VITRO SENSITIVITY OF MYCO. AND ASSOCIATED BACT.


### Table (1)
Recovery Rate of Mycoplasmas and associated bacteria from chicken and Turkey and duck samples

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Chicken</th>
<th></th>
<th>Turkey</th>
<th></th>
<th>Duck</th>
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<tbody>
<tr>
<td></td>
<td>No.</td>
<td>No.</td>
<td>Percentage</td>
<td>No.</td>
<td>No.</td>
<td>Percentage</td>
</tr>
<tr>
<td></td>
<td>Examined</td>
<td>Positive</td>
<td>%</td>
<td>Examined</td>
<td>Positive</td>
<td>%</td>
</tr>
<tr>
<td>M. gallisepticum</td>
<td>50</td>
<td>12</td>
<td>24</td>
<td>50</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>M. meleagris</td>
<td>50</td>
<td>--</td>
<td>--</td>
<td>50</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>M. anatis</td>
<td>50</td>
<td>--</td>
<td>--</td>
<td>50</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>M. gallinarum</td>
<td>50</td>
<td>--</td>
<td>--</td>
<td>50</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>M. iners</td>
<td>50</td>
<td>--</td>
<td>--</td>
<td>50</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>A. laidlawii</td>
<td>50</td>
<td>--</td>
<td>--</td>
<td>50</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>A. avanthum</td>
<td>50</td>
<td>--</td>
<td>--</td>
<td>50</td>
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<td>--</td>
</tr>
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<td>E. coli</td>
<td>50</td>
<td>20</td>
<td>40</td>
<td>50</td>
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<td>S. gallinarum pullorum</td>
<td>50</td>
<td>7</td>
<td>14</td>
<td>50</td>
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<td>Steph. aureus</td>
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<td>5</td>
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<td>Pseud. aeruginosa</td>
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<td>12</td>
<td>24</td>
<td>50</td>
<td>15</td>
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### Table (2)

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Tetraacycline</th>
<th>Amoxicillin</th>
<th>Erythromycin</th>
<th>Nalidixic acid</th>
<th>Nitrofurantoin</th>
<th>Spectinomycin</th>
<th>Tetracycline 0.1 mg/mL</th>
<th>Doxycycline 0.05 mg/mL</th>
<th>Josamycin 0.05 mg/mL</th>
<th>Aztreonam 100 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 µg</td>
<td>10 µg</td>
<td>30 µg</td>
<td>30 µg</td>
<td>30 µg</td>
<td>150 µg</td>
<td>20 µg</td>
<td>100 µg</td>
<td>100 µg</td>
<td>100 µg</td>
</tr>
</tbody>
</table>

### Resistant Strains

- *Streptococcus faecalis*
- *Staphylococcus aureus*
- *Escherichia coli*
- *Proteus vulgaris*
- *Klebsiella pneumoniae*
- *Haemophilus influenzae*
- *Moraxella catarrhalis*
- *M. galliseptica*
### Table (3)

Results of Hatching Rate and Recovery Rate of Mycoplasmas from Antibiotic treated turkey eggs

<table>
<thead>
<tr>
<th>Antibiotic drugs</th>
<th>Number of Eggs</th>
<th>Hatched Poult Recovery Rate of Mycoplasma from</th>
<th>Dead Embryos</th>
<th>Hatched Poult after two- week age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inoculated</td>
<td>Alive at days</td>
<td>Hatchability Percentage %</td>
<td>No.exam.</td>
</tr>
<tr>
<td>Josamycin*</td>
<td>50</td>
<td>9</td>
<td>41 35 33</td>
<td>30</td>
</tr>
<tr>
<td>Doxycycline**</td>
<td>50</td>
<td>9</td>
<td>35 28 18</td>
<td>15</td>
</tr>
<tr>
<td>Tylosine-tartarate</td>
<td>50</td>
<td>8</td>
<td>32 25 15</td>
<td>12</td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td>10</td>
<td>30 25 12</td>
<td>9</td>
</tr>
</tbody>
</table>

* Josamycin = alphucine-20% Verbac Lab., France.
** Doxycycline = Vibraet Pfizer lab., U.S.A.