قسم : أمراض الدواجن .
كلية : الطب البيطرى - جامعة أسيوط .
رئيس القسم : أ.د / ابراهيم حسن سكر .

اختبار حساسية عترات الميكوبلازما والبكتريا المصاحبة لها والمعزولة من الدجاج والرومي والبط بصعيد مصر العليا

ابراهيم سكر ، عادل سليمان ، صلاح موسى ، محسن الدمرداش

تم تجميع مسحات من الحنجرة والجيوب الأنفية والاكياس الهوائيه من الدجاج والرومي والبط وقد ثبت وجود ميكوبلازما جاليسبتكم بنسبة ٢٤ - ٣٢٪ في الدجاج والرومي بالترتيب وم. ميلياجريس في الرومي بنسبة ٣٠٪ بينما تم عزل م.أناتس (١٤٪) ، أ. ليدلاوى (٢٨٪) ، أ.اكزايثم (٣٢٪) من البط وكانت هذه العترات مصاحبة للميكروب القولوني ، ميكروب السيد ومانس والميكروب العنقودى الذهبي وميكروب السالمونيلا بللورم . وقد أوضح البحث أن هذه العترات عالية الحساسية للجوراميسين ومتوسطة الحساسية للينكومايسين وغير حساسة لبقية المضادات الحيويه المستخدمه .

وباستخدام طريقة تغطيس بيض الرومي المصاب بالميكوبلازما في تركيزات مختلفة من المضادات الحيويه فقد ثبت أن عقار الجوراميسين تسبب في خفض نسبة وجود الميكروب وتلاه الدوكسي سيكلين ثم جاء بعد التايلان .

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IN-VITRO SENSITIVITY OF MYCOPLASMAS AND ASSOCIATED BACTERIA ISOLATED FROM CHICKENS & TURKEYS AND DUCKS AT THE AREA OF UPER EGYPT

(With 3 Tables)

By
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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. axanthum (32%) were recovered from ducks. These isolates were found in association with E. coli, Pseud. aeuroginosa, Staph. aureus and S. gallinarum pullorum. All isolates showed high sensitivity to josamycine, moderate sensitivity to lincomycine and low variable sensitivity to other antibiotics. Incubated turkey-eggs from infected farm showed good elemination of mycoplasma after dippoing in josamycine solution, while inferior results were obtained with doxycyline and tylosin-tartarate 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-sacculitis in turkey poults, late incubation mortality and poor growth rate due to M. meleagridis were reported by GHAZIKHANIAN and YAMAMOYO (1974). Air-sacculitis and ascitis in ducks due to M. anatis, M. gallinarum, M. iners, A. laidlawii and A. axanthum were described by ROBERTS, (1964), KARPAS, (1969), EL-EBEEDY, (1976), FAWZIA, (1976) and SOLIMAN, (1985). Complicated air-sacculitis in turkey- poults infected with M. meleagridis and E. coli were recorded by MOHAMED et al. (1970). Also E. coli, Staph. aurus, Pseud. aeuroginosa and S. gallinerum pullorum were recovered from upper and lower respiratory organs by BERGMAN, et al. (1980) and KIBENGE 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-tartarate. 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. auroginosa, they reported the greater resistance of various E. coli strains and Pseud. aeuroginosa to erythromycin, neomycin, sulphonamides and Nitrofurantion. An increased rate of hatchability

ar[illegible]low recovery rate of mycoplasmas in treated turkey- eggs by egg- injection or egg- dipping by using various antibiotics were reported by (YAMAMOTO and BIGLAND, 1966 & ELFTERESCU, et al. 1972 & MACAPES, et al. 1976, 1977 & ELMAHI and HOFSTAD, 1978).

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 discribed 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, S-S agar and Crystal-Violte-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 Ugjhon Company, U.S.A. were used, while sensitivity discs for josamycin (0.05 mg/ml) were prepared from "alplucine 20%" produced by Virbac-laboratories, France, and "Doxycycline" discs were prepared from "Vibravet" 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 mycoplasma:

A total of 200 turkey-eggs were obtained from El-Wadi El-Gadid turkey farm the had 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.", Vibravet 50 p.p.m." and "Tylosine-tartarate 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 isulation 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.

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 paralle with observations of KARPAS and FABRICANT (1969), FAWZIA (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-tartarate,Nitrofurantion, Neomycin and oxytetracycline. These results were agreed with KLEVEN, et al. (1971), ELMAHI, et al. (1978), HAMDY, et al. (1980), YOON, et al. (1981), SOLIMAN (1982), EL-BAKRY (1983), and SOLIMAN (1985).

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

Results of senitivity test points to the need of periodical testing of bacteial isolates to detect their sensitivity to the available intimicrobial agents in order to choose the more effective drugs for using in the prophylactic and therabutic programs.

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

Isolates	Chicken			Turkey			Duck		
	No. Examined	No. Positive	Percentage %	No. Examined	No. Positive	Percentage %	No. Examined	No. Positive	Percentage %
M. gallisepticum	50	12	24	50	16	32	50	--	--
M. meleagridis	50	--	--	50	15	30	50	--	--
M. anatis	50	--	--	50	--	--	50	8	16
M. gallinarum	50	--	--	50	--	--	50	12	24
M. iners	50	--	--	50	5	10	50	7	14
A. laidlawii	50	--	--	50	6	12	50	14	28
A. awanthum	50	--	--	50	--	--	50	16	32
E. coli	50	20	40	50	12	24	50	8	16
S. gallinarum pullorum	50	7	14	50	3	6	50	3	6
Staph. aureus	50	5	10	50	3	6	50	2	4
Pseud. aeuroginosa	50	12	24	50	15	30	50	10	20

Table (2)

Results of in-vitro sensitivity of Mycoplasmas and associated Bacteria

Isolate strains / Antibiotic discs.	Josamycine 0.05 mg/ml.	Doxycycline 0.1 mg/ml.	Tylosine-Tartarate 0.1 mg/ml.	Spectinomycin 100 ug	Lincomycin 20 ug	trythromycin Erythromycin 15 ug	Nitrofurantion 30 ug	Oxytetracycline 30 ug	Neomycin 30 ug	Chloramphenocol 30 ug	Ampicillin 10 ug	Tetracycline 30 ug
M. gallisepticum	+++	+++	-	‡	‡‡	‡	-	+	+	-	+	+
M. meleagridis	‡‡	+	-	+	‡	‡	-	+	-	-	+	+
M. anatis	‡‡‡	‡	+	‡	‡	+	+	‡	-	‡	‡	+
M. gallinarum	‡‡‡	‡	+	‡	‡	‡	+	+	+	‡	+	+
M. iners	‡‡‡	‡	+	+	‡	‡	+	+	+	+	+	+
A. laidlamii	‡‡‡	‡	+	+	‡‡	‡	-	-	‡	‡	‡	‡
A. axanthum	‡‡‡	‡	+	‡	+	‡	‡	+	‡	‡	‡	‡
E. coli	‡‡‡	‡	-	-	‡	‡	‡	‡	+	‡	+	+
S. gallinarumpollorum	‡‡	‡	-	-	‡‡	‡	‡	+	-	-	+	-
Staph. gurus	‡‡‡	+	-	-	‡‡	‡	‡	+	‡	-	‡	+
Pseud. aeuroginosa	‡‡‡	-	-	-	‡	‡	‡	+	+	+	‡	-

++++ highly sensitive & +++ sensitive & ++ Moderately sensitive & + weakly sensitive and - Resistant.

Table (3)
Results of Hatching Rate and Recovery Rate of Mycoplasmas from Antibiotic treated turkey eggs

Antibiotic drugs	Number of Eggs:					Hatched Poults		Recovery Rate of Mycoplasma from				
	Inoculated	Infertile	Alive at day: 7	15	21	Number	Hatchability Percentage %	Dead Embryos No.exam.	No.Pos.	Hatched Poults after two- week age No.exam.	No.with air-sac	No.Pos.
Josamycin*	50	9	41	35	33	30	73	11	2	30	5	8
Doxycycline**	50	9	35	28	18	15	36	26	14	15	8	12
Tylosine-tartarate	50	8	32	25	15	12	28	30	28	12	9	11
Control	50	10	30	25	12	9	22	31	28	9	7	9

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