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IN VITRO SENSITIVITY TO ANTIBIOTICS OF SWEDISH STRAINS OF MYCOPLASMA GALLISEPTICUM

By

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Respiratory diseases of poultry are unanimously regarded as a major economic hazard to modern poultry husbandry, in most countries. In the USA, Great Britain, and most continental European countries with a heavily industrialized poultry husbandry both virus diseases such as Newcastle disease, infectious bronchitis, and infectious laryngotracheitis, and mycoplasmosis are incriminated as the main causes of loss due to respiratory infection.

In Sweden these matters are somewhat different due to the rare occurrence or even non-existence of the virus diseases just mentioned and also of the massification of birds in restricted areas so characteristic to the major poultry industry nations. Nevertheless, an aggravation of poultry respiratory diseases with respect to both incidence and character has long been noted even in this country. Such a trend has been testified to by *Lindgren* (1955) and *Bakos & Karlsson* (1962), in a report of an explorative investigation into the incidence of *Mycoplasma* infection, proved this infection to be a cause of chronic respiratory outbreaks in this country. They also showed that the Swedish strains were essentially identical with American strains available for comparison with respect to antigenic and serologic properties. *Andersson* (1964) recently reported on therapeutic experiments in mycoplasmosis in connection with disease outbreaks in south Sweden, and there is ample evidence that this disease must now be regarded as a serious reality to poultry husbandry even in

Sweden. An account of mycoplasmosis with reference to conditions pertinent to the Nordic countries has been given by *Marthedal* (1962).

Ever since the establishment of the sensitivity to broad spectrum antibiotics (tetracyclines) of *Mycoplasma gallisepticum* these, and more recently even other antibiotics such as erythromycin and tylosin have been widely used in both preventive and curative treatment of *Mycoplasma* infection in poultry. This is true even when programmes for the creation of *Mycoplasma*-free flocks are applied in mycoplasmosis control, and antibiotic treatment also is an important tool in the efforts to control mycoplasmosis by breaking the disease transmission chain via the hatching egg which is unanimously claimed to be the most important route of infection.

Whichever method of control is chosen, the need for effective antibiotics is apparent. According to findings by *Domermuth* (1960) drug resistance must be taken into consideration in *Mycoplasma*, and this fact stresses the desirability for a choice between several different antibiotics. *Yamamoto & Adler* (1956) examined eight chick strains of *Mycoplasma gallisepticum* for sensitivity to different antibiotics, and proved them all to be highly sensitive to tetracyclines. The experiments reported in this paper were carried out to investigate if the strains isolated in Sweden by *Bakos & Karlsson* agree with strains from the American continent even with respect to sensitivity to antibiotics.

MATERIAL AND METHODS

The *Mycoplasma* strains tested were 9 available out of 11 strains isolated by *Bakos & Karlsson*.

The antibiotics used were those listed in *Table 1* kindly supplied by representatives of the drug industry as: Tylan and Erythromycin, *Ely Lillie* (*Ercopharm AG*, Copenhagen), Achromycin and Aureomycin, *Lederle* (*Kemi-Intressen AB*, Stockholm), Kanamycin sulphate, Syntodecin, Alfacillin, Cetacillin and Doctacillin, *Astra* (*AB Astra*, Södertälje), Terramycin, *Pfizer* (*Pfizer AB*, Näsbypark, Stockholm) and Streptomycin and Dihydrostreptomycin, *Kabi* (*AB Kabi*, Stockholm).*

Dihydrostreptomycin was tested in 8 strains only due to the fact that one strain had died by the time this antibiotic was included in the experiment.

* My thanks are due to the firms and companies for kindly supplying the necessary antibiotics.

The methods used were the same as described by *Yamamoto & Adler*. PPLO broth (Difco) with an addition of 20 per cent horse serum was used as the test medium. It was reduced by half its volume by evaporation, and the original volume and salt content were restored by aid of the different antibiotic dilutions in distilled water. Inoculation was made with one drop (approx. 0.05 ml) of a 48 hours broth culture of the *Mycoplasma* strains. After 36 hours of incubation at 37°C one loopful of each dilution was smeared into PPLO-agar plates with 20 per cent added horse serum. The plates were incubated for 72 hours and examined for *Mycoplasma* colonies. Readings were restricted to bactericidal effect, the bacteriostatic effect being very difficult to appreciate owing to the slow and irregular growth of *Mycoplasma gallisepticum*. Dilutions permitting no growth were considered as showing the lethal concentration of an antibiotic.

RESULTS AND DISCUSSION

Results are shown in *Table 1*.

Most of the strains tested show a high sensitivity to terramycin and achromycin which is in agreement with the finding of *Yamamoto & Adler*. Erythromycin, shown by the same workers to have a comparable effect with aureomycin and streptomycin showed a remarkably low effect in the present experiment. Tylosin was not available in 1956 but has received considerable attention in the control of mycoplasmosis in the USA during recent years. It is shown by my experiments to have the highest *in-vitro* effect of the antibiotics tested. The other antibiotics included in my experiments were chosen with the hope of finding new compounds with high effect against *Mycoplasma*, none of them, to my knowledge, having been tested earlier for such effects. Syntodecin and kanamycin, showing an acceptable effect against 6 of the 9 strains tested, may possibly prove useful as new tools in mycoplasmosis control. In contrast, the semisynthetic penicillin compounds showed no effect whatsoever against the tested *Mycoplasma* strains.

Even though the present investigation should be taken to indicate far reaching agreement between ours and S 6 type *Mycoplasma* strains from other parts of the world with respect to the examined properties, dissimilarities have also been illustrated. It is a well-known fact, that antibacterial spectra of antibiotics

Table 1. Bactericidal activity of different antibiotics against 9 strains of *Mycoplasma gallisepticum*.

Antibiotic	Minimum bactericidal concentration in micrograms/ml					
	0.1-1.0	1.1-10.0	10.1-100.0	100.1-500.0	500.1-1000.0	> 1000.0
Tylosin	2	1	4	2		
Achromycin		2	7			
Kanamycin		2	4	3		
Terramycin	1	1	4		3	
Syntodecin	1	1	4		3	
Aureomycin			6		3	
Streptomycin	2		1		5	
Erythromycin			1	7	1	
Dihydrostreptomycin	1		1		2	4
Alfacillin				2		7
Cetacillin						9
Doctacillin						9

are not constant but vary from time to time and in different environments. Findings in one country, therefore, must not uncritically be expected to be valid for any other part of the world. Also, it must be born in mind that the results of the *in-vitro* experiments reported in this paper may not unreservedly be thought to be applicable *in-vivo*. The suggestions as to a possible development of the therapeutical arsenal against mycoplasmosis offered by the investigation, therefore, must so far aim only at investigations into the prophylactic and therapeutic *in-vivo* effect on *Mycoplasma* infection.

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SUMMARY

The *in-vitro* sensitivity of 9 different strains of *Mycoplasma gallisepticum* has been tested using 12 different antibiotics including tetracyclines, erythromycin and tylosin. The latter antibiotic showed the highest effect followed by achromycin and terramycin. Two antibiotics with a high *in-vitro* effect against *Mycoplasma* were found in kanamycin and syntodecin.

ZUSAMMENFASSUNG

Die in-vitro Empfindlichkeit in schwedischen Stämmen von *Mycoplasma gallisepticum* gegenüber Antibiotika.

Die *in-vitro* Empfindlichkeit in 9 verschiedenen Stämmen von *Mycoplasma gallisepticum* ist unter Anwendung von 12 verschiedenen Antibiotika, Tetracycline, Erythromycin und Tylosin umfassend, untersucht worden. Das letztgenannte Antibioticum zeigte den grössten Effekt, darauf folgten Achromycin und Terramycin. Zwei Antibiotika mit einem grossen *in-vitro* Effekt gegenüber *Mycoplasma* wurden in Kanamycin und Syntodecin gefunden.

SAMMANFATTNING

Känsligheten in-vitro emot antibiotika hos svenska stammar av *Mycoplasma gallisepticum*.

Känsligheten *in-vitro* för antibiotika hos 9 stammar av *Mycoplasma gallisepticum* har undersökts för 12 olika antibiotika inklusive tetracykliner, erythromycin och tylosin. Det senare antibiotikat visade den högsta effekten följd av achromycin och tylosin. Två antibiotika med en hög *in-vitro* effekt påträffades i kanamycin og syntodecin.

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