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INHIBITORY AND MYCOPLASMACIDAL CONCENTRATIONS OF SOME ANTIBIOTICS ON STRAINS OF MYCOPLASMA MYCOIDES SUBSP. MYCOIDES SC: THE CAUSATIVE AGENT OF CONTAGIOUS BOVINE PLEUROPNEUMONIA

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The minimum inhibitory and mycoplasmacidal concentrations of five antibiotics commonly used in the treatment of bovine respiratory infections to eight strains of Mycoplasma mycoides subsp. mycoides SC are reported. Enrofloxacin (a fluroquinolone) and Tylosin showed marked inhibitory activity against the vaccine (T_{1-44}) and field strains tested. The minimum inhibitory (MICs) and mycoplasmacidal concentrations (MMCs) for these two antibiotics ranged from 0.01 - 0.06 μg/ml and 0.13 - 0.50 μg/ml for Enrofloxacin compared with values of 0.03 - 0.06 μg/ml and 0.13 - 4.00 μg/ml for Tylosin. Doxycycline and Lincomycin showed weaker inhibition with values of 0.06 - 2.00 μg/ml and 0.13 - 2.00 μg/ml, respectively. Lincomycin showed weak mycoplasmacidal activity (0.25 - 8.00μ/ml) similar to doxycycline (0.50 - 16.00 mg/ml).

It is hoped that these in-vitro base line MIC & MMC values will assist in the choice of antibiotics for treating bovine respiratory diseases in which M. mycoides subsp. mycoides SC usually occurs solely or mixed with other pathogens.

Key words: Mycoplasma mycoides subsp mycoides SC, antibiotics, contagious bovine pleuropneumonia.

INTRODUCTION

Mycoplasma mycoides subsp. mycoides SC belongs to a group of six closely related mycoplasmas known as the "mycoides cluster" which share many biochemical, immunological and genetic characteristics (Cottew et al., 1987). Members of this cluster cause disease in cattle, sheep and goats (MacOwan,

1983, Ferronha et al., 1988, Nunes-Pestica et al., 1990). Of these diseases, Contagious Bovine Pleuropneumonia (CBPP) is considered by the Office des International Epizooties (OIE) as the ost significant in countries where these diseases occur (OIE, 1995).

CBPP is endemic in most parts of Africa and its occurrence in hitherto free zones of Italy, Spain, France and Portugal has been described (Nicholas and Palmer, 1994; Egwu, et al., 1996).

Sensitive and specific immunodiagnostic tests (Bashiruddin et al., 1994) and immunoprophylaxis, coupled with an efficient slaughter policy for sero-positive animals are measures recommended for the eradication of the disease. A polymerase chain reaction has been developed using mycoides specific (451) primers involving DNA extraction, aplification electrophoresis, digestion of the PCR product with Asni and staining with ethidium bromide (Nicholas et al., 1994).

Many measures have been proposed for the control and eradication of CBPP (OIE, 1993). However, most countries of Africa where CBPP is endemic are incapable of implementing the test and slaughter policy because of the huge financial costs involved. As a result, treatment of affected animals (usually at the farmer's request) and vaccination are usually resorted to during outbreaks (Egwu, Personal observations).

Therefore, this paper reports on the minimum inhibitory (MICs) and mycoplasmacidal (MMCs) concentrations of some antibiotics commonly used in the treatment of bovine respiratory infections in order to assess their efficacy (invitro) against Mycoplasma mycoides subs. mycoids SC (the causal agent of CBPP) which frequently occurs in combination with other respiratory pathogens (Egwu, personal observation).

Assays of the MICs were performed in sterile covered micro-titre plates (R) (Sigma - Aldrich, England) based on the metabolism inhibition test (MIT) with a slight modification of the method described by Senterfit (1983) using glucose as the substrate and phenol red as the indicator of colour change. Briefly, each titrated test culture for use in the test proper was diluted to contain 1000CCU/0.2ml (a reasonably likely field inoculum). Two-fold serial dilutions of the stock antibiotic (64 μg/ml) was performed in 100 μl volumes in micro-titre plates using Eaton's medium as diluent, except for Enrofloxacin for which dilution was continued in the same row of a second microtitre when no growth was observed in the last well of the first plate. This was followed by the addition of 100 μl of the adjusted test strain to give a total volume of 0.2ml in each well, each containing 100 μl of antigen and the test volume served as the antigen control while control wells contained 200νl of the test medium. The plates were incubated for 4-5 days. The initial MICs were recorded as soon as the inoculum control wells showed a colour change with a drop in pH of 0.5. The final MICs were read when the colour change compared to control wells was fully expressed. The MMC were determined by withdrawing 10 μl from wells along a row showing inhibition of colour change into fresh 2ml medium in sterile bijou bottles. This 1:200 dilution reduced the antibiotic concentration below the MIC(s) of most of the tested strains. The incubation was similarly performed and tests were redas previously described for the MICs.

MATERIALS AND METHODS

Eaton's medium without antibiotics described in the Central Veterinary Laboratory Agency's handbook on mycoplasmas was used for the subculture, maintenance, titration and assay of the test mycoplasmal strains. The types and origin of the eight test strains as well as their passage levels prior to the antibiotic assay are shown in Table 1. Also the sources of the five different antibiotics used in this study are shown in the footnote of Table 2. Stock antibiotic concentrations were prepared in sterile distilled water at a working concentration of 64 μg/ml, filtered through a 200nm millipore filter, stored at -20^{0}C and used within a week of preparation. Where the potency of the antibiotic used was not 100%, the actual working concentration was adjusted using the formula: Required weight = 1000/potency in μg/ml x volume required as described by Andrews and Wise (1978).

Prior to titration, all test strains used were checked for purity by the polymerase chain reaction (PCR) using cluster specific (351) and mycoides specific primers.

Table 1. Strains, origin and passage levels prior to antibiotic assay.

Strains of **M. mycoides** subsp. **mycoides** SC	Origin	Number of further passages prior to assay
* PG1 (NCTC 10114)	NK	2
** KH_3J	Sudan (Juba)	3
V5	Australia	2
375	Botswana	3
** $T1_{44}$	Tanzania	1
6529	Portugal	2
192	Italy	3
Gladysdale	Australia	2

NK = Not known
* Pype strains
** Vaccine strains

RESULTS

The MICs and MMCs of eight strains of M. mycoides subsp. mycoides SC to five antibiotics are shown in Table 1. Marked inhibitory activity against the vaccine and field strains tested was observed for Enrofloxacin and Tylosin. Slight variations were observed amongst strains in their MIC and MMC values. Enrof loxacin had the lowest MIC and MC with values of 0.01 - 0.06 μg/ml and 0.50 μg/ml respectively compared with Tylosin with values of 0.03 - 0.06 μg/ml and 0.13 - 4.00 μg/ml, respectively. Doxycycline and Lincomycin were less inhibitory with values of 0.06 - 2.00 μg/ml and 0.13 - 2.00 μ/ml, respectively, whilst Lincomycin showed weak mycoplasmacidal activity with values of 0.25 - 8.00 μg/ml similar to Doxycycline with MMC values of 0.50 - 16.00 μg/ml, respectively. Ampicillin

showed no inhibitory or mycoplasmacidal activity within the range of values tested.

Table 2: Inhibitory and mycoplasmacidal concentration of eight strains of M. mycoides subsp. mycoides SC to five antibiotics.

	Concentrations in μg/ml				
Strains of M. mycoides Sc	Enrofloxacin	Tylosin	Doxycycline	Lincomycin	Ampicillin
PG1 (NCTC 10114)	0.03 (0.06)	0.06 (0.13)	0.25 (0.50)	0.25 (0.25)	64 (NT)
KH3J	0.03 (0.13)	0.50 (1.00)	1.00 (1.00)	1.00 (2.00)	64 (NT)
V5	0.01 (0.50)	0.03 (2.00)	0.06 (4.00)	0.13 (4.00)	64 (NT)
375	0.03 (0.06)	0.06 (0.25)	0.13 (1.00)	0.13 (0.25)	64 (NT)
T144	0.10 (0.05)	0.03 (4.00)	0.06 (2.00)	0.50 (4.00)	64 (NT)
6529	0.01 (0.50)	0.06 (2.00)	0.13 (16.00)	0.25 (8.00)	64 (NT)
192	0.06 (0.13)	0.13 (0.25)	0.13 (0.50)	0.50 (2.00)	64 (NT)
Gladysdale	0.01 (0.06)	0.50 (1.00)	2.00 (4.00)	1.00 (2.00)	64 (NT)
Range of values	0.01–0.06 (0.13–0.50)	0.03–0.06 (0.13–4.00)	0.06–2.00 (0.50–16.00)	0.13–2.00 (0.25–8.00)	64 (NT)

( ) Figures in brackets are MMCs
NT = Not tested
Enrofloxacin R (Bayer, Germany)
Tylosin tartrate (Sigma-Aldrich, England)
Doxycycline HCl "
Lincomycin "
Ampicillin sodium "

DISCUSSION

In vitro antibiotic susceptibility studies have been reported for a variety of mycoplasma strains from ruminants (Terlaak et al., 1993, Ball et al., 1995), but no reports exist to the best of our knowledge for M. mycoides subs. mycoides SC.

Purposeful or indiscriminate use of antibiotics directed against respiratory or other infectious diseases of cattle may have direct or indirect effects on other offending pathogenic mycoplasmas such as M. mycoides subsp. mycoides SC, particularly in countries where CBPP is endemic. Clearly, there is a need for base line information on the inhibitory and mycoplasmacidal effects of some of these

commonly used drugs particularly on M. mycoides subsp. mycoides SC and comparison of these values with those reported for other bovine mycoplasmas.

The results obtained in this present study have shown the superiority of Enrofloxacin (a fluroquinolone) over Tylosin concerning inhibitory and mycoplasmacidal activity against M. mycoides subsp. mycoides SC in vitro. These values for Enrofloxacin were slightly lower than those reported for the same drug against M. bovis strains isolated from bovine mastiitic gland by Ball and coworkers (1995). It may well be necessary to determine the in vivo concentration of this drug (Enrofloxacin) in the lungs, although Malbe and others (1996) reported peak blood concentrations of 5.4mg/1 at 2h following i/v administration at a dose rate of 5mg/kg in six mid lactating cows indicating good distribution of the drug. Terlaak and others (1993), using a wide variety of field and type strains of M. bovis, reported MIC values for Tylosin similar to those reported in this study. Also the MIC values (0.13 - 2.00 μg/ml) indicated for Lincomycin in our present study parallel those reported by Terlaak and others (1993) and Ball and coworkers (1995). Doxycycline (a newer analogue of the tetracyclines) was found in this present study to possess lower MIC but higher MMC values than Lincomycin, whilst, in contrast, resistance of M. bovis strains to the older tetracyclines (such as Oxytetracycline) was reported by Terlaak and others (1993).

The present practice of treating affected animals with CBPP in most developing countries of Africa, contrary to OIE recommendations, offers no better hope for the future, except when the problems associated with the present field vaccines (such as frequent vaccine break down, poor immunogenicity, multiplicity of field vaccines) are clearly addressed. Perhaps research into the production of recombinant DNA vaccine may offer a better alternative immunoprophylaxis. Nevertheless, one of the consequences of treating affected cases of CBPP is that it can result in the perpetuation of chronicity (lungers) in affected herds. It seems obvious that African countries where CBPP is endemic will just have to live with the disease for the time being until a more protective vaccine is available.

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DELOVANJE INHIBICIONIH I MIKROPLAZMACIDNIH KONCENTRACIJA NEKIHANTIBIOTIKA NA RAZLIČITE SOJEVE UZROČNIKA KONTRAGIOZNE PLEUROPNEUMONIJE GOVEDA: MYCOPLASMA MYCOIDES SUBSP. MYCOIDES

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SADRŽAJ

U radu su opisani minimalni inhibitorni i mikoplazmacidni efekti različitih koncentracija pet antibiotika koji se koriste u terapiji respiratornih infekcija goveda sa osam sojeva Mycoplasma mycoides subsp. mycoides SC. Enrofloxacin (fluroquinolone) i Tylosin su pokazali izrazite inhibitorne efekte i na vakcinalni soj i na divlje sojeve koji su ispitani. Minimalna inhibitorna (MICs) i minimalna mikoplazmicidna koncentracija ova dva antibiotika se kretala od 0.01 - 0.06 μ/ml i 0.13 - 0.50 μg/ml Enrofloxacina, odnosno 0.03 - 0.06 μg/ml i 0.13 - 2.00 μg/ml Tilozina, po redosledu. Mikoplazmicidna aktivnostinkomicina je bila slaba (0.25 - 8.00 μg/ml), a slično je ustanovljeno i za doxycycline (0.50 - 16.0 μg/ml). Inhibitorna aktivnost ova dva antibiotika je takođe bila slaba.

Ustanovljene bazne MIC i MMC vrednosti mogu da pomognu pri izboru antibiotika u terapiji respiratornih oboljenja goveda, gde se inače često pojavljuju M. mycoides SC u mešanoj infekciji ili samostalno.