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Assessing the In Vitro Effectiveness of Antimicrobials against *Mycoplasma mycoides* subsp. *mycoides* Small-Colony Type To Reduce Contagious Bovine Pleuropneumonia Infection

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In vitro minimum inhibitory concentrations were determined for 21 antimicrobials against 41 isolates of *Mycoplasma mycoides* subsp. *mycoides* small-colony type, the cause of contagious bovine pleuropneumonia. Of the antimicrobials used most widely in Africa, oxytetracycline and tilmicosin were effective, while the isolates were resistant to tylosin. These results provide a baseline for monitoring antimicrobial resistance.

Contagious bovine pleuropneumonia (CBPP), an Office International des Epizooties-listed disease, is caused by *Mycoplasma mycoides* subsp. *mycoides* small-colony type (MmmSC). In Africa the disease is widespread and threatens to extend into areas where it is currently not found. Control of the disease is difficult, but Botswana, Portugal, and Italy have relatively recently eradicated the disease. These successful campaigns relied on effective diagnosis, slaughter, and compensation methods. The only other method is vaccination with a live attenuated strain, T_1/44, which offers short-term immunity, but can cause adverse reactions and even CBPP itself (6). Development of alternative vaccines has been unsuccessful, often resulting in exacerbation of disease (9).

Treatment of affected cattle with antimicrobials is officially discouraged, although in many African countries it is widely practiced (5). Previously it has been reported that antimicrobial treatment may alleviate the clinical signs, but not prevent the spread of infection, and may favor the creation of chronic carriers (10). However, one recent recommendation was that research should be conducted on the activity of new antimicrobials in controlling CBPP (3).

A small trial in Namibia demonstrated that treatment of CBPP-affected cattle with Advocin reduced the spread of CBPP to healthy cattle (4). If antimicrobials are to be considered an additional method for CBPP control, then several factors have to be considered, including selection of the most suitable antimicrobial, withdrawal periods to avoid antimicrobial residues in milk and meat, and the possible development of antimicrobial resistance by MmmSC.

This study investigates the in vitro effectiveness of 21 antimicrobials against 41 isolates of MmmSC (see Table 2). This also provides baseline information for future monitoring for antimicrobial resistance. The availability of recent MmmSC isolates was limited; however, 27 were isolated in Africa between 1996 and 2004. The remaining MmmSC isolates consisted of older strains and eight strains that had previously been tested against five of the antimicrobials used in this study (1). The field isolates had been minimally passaged in Eaton's broth medium (7) and stored at −70°C until required. Species identification was confirmed by standard mycoplasma identification techniques (8) and by PCR (2).

The MIC methods used were described previously (1). With the exception of tylosin (TYL), the antimicrobials were all obtained in pure form from Trek Diagnostic Systems (East Grinstead, West Sussex, United Kingdom) at specified concentrations on microtiter Sensititre plates. The plates were designed for use at a final volume of 200 μl and provided doubling dilutions of danofloxacin (DAN), oxytetracycline (OXYTET), tilmicosin (TIL), spectinomycin (SPT), florfenicol (FLO), enrofloxacin (ENRO), cephalothin (CEF), and gentamicin (GEN) from 0.06 μg/ml to 64 μg/ml. For amikacin (AMK), chloramphenicol (CHL), ciprofloxacin (CIP), clindamycin (CLI), erythromycin (ERY), lincomycin (LIN), nalidixic acid (NAL), norfloxacin (NOR), rifampin (RIF), streptomycin (STR), and tobramycin (TOB), doubling dilutions from 0.12 μg/ml to 32 μg/ml were used, except the dilutions at 4 μg/ml and 16 μg/ml were omitted. For trimethoprim-sulfamethoxazole (SXT), combined concentrations were 0.12/2.37, 0.25/4.75, 0.5/9.5, 1/19, 2/38, 8/152, and 32/608 μg/ml, respectively. TYL (Sigma, Poole, United Kingdom) was diluted according to Clinical and Laboratory Standards Institute (CLSI) (formerly NCCLS) guidelines (11) to give a final antimicrobial range from 0.03 μg/ml to 64 μg/ml of active ingredient. Controls without an antimicrobial were also included, and all samples were tested in duplicate.

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TABLE 1. Summary of MIC and MMC values for 41 isolates of *Mycoplasma mycoides* subsp. *mycoides* small-colony type

Antimicrobial	MIC (μg/ml)			MMC (μg/ml)		
	Range	50%	90%	Range	50%	90%
TIL	<0.06–0.12	<0.06	<0.06	0.12–16.00	0.25	2.00
OXYTET	<0.06–0.50	<0.06	0.12	4.00–64.00	16.00	32.00
ERY	<0.12–0.25	<0.12	0.25	1.00–>32.00	8.00	>32.00
ENRO	0.12–0.50	0.12	0.25	2.00–8.00	4.00	8.00
CLI	<0.12–0.50	0.25	0.25	1.00–32.00	8.00	32.00
DAN	0.12–0.50	0.25	0.25	4.00–32.00	8.00	16.00
CIP	<0.12–0.50	0.25	0.50	8.00–32.00	8.00	8.00
LIN	<0.12–2.00	0.50	1.00	8.00–>32.00	8.00	>32.00
CHL	0.25–2.00	1.00	2.00	32.00–>32.00	32.00	32.00
FLO	0.25–8.00	1.00	2.00	8.00–>64.00	16.00	32.00
NOR	1.00–>32.00	2.00	8.00	32.00–>32.00	32.00	>32.00
SPT	2.00–16.00	8.00	16.00	64.00–>64.00	>64.00	>64.00
TYL	0.12–>64.00	16.00	64.00	NT[a]	NT	NT
TOB	8.00–>32.00	32.00	32.00	>32.00–>32.00	>32.00	>32.00
GEN	16.00–64.00	32.00	64.00	>64.00–>64.00	>64.00	>64.00
NAL	8.00–>32.00	>32.00	>32.00	>32.00–>32.00	>32.00	>32.00
CEF	16.00–>64.00	>64.00	>64.00	>64.00–>64.00	>64.00	>64.00
STR	32.00–>32.00	32.00	32.00	>32.00–>32.00	>32.00	>32.00
RIF	32.00–>32.00	>32.00	>32.00	>32.00–>32.00	>32.00	>32.00
AMK	>32.00–>32.00	>32.00	>32.00	>32.00–>32.00	>32.00	>32.00
SXT	>32/608–>32/608	>32/608	>32/608	>32/608–>32/608	>32/608	>32/608

[a] NT, not tested.

For the MIC test, the MmmSC isolates were grown in 3.5 ml of Eaton's broth medium for 48 h at 37°C. The inoculum was standardized to approximately 10^8 CFU/ml (1). One hundred ninety microliters of medium was dispensed into the Sensititre plate, and 10 μl of inoculum was added. As tylosin was initially omitted from the Sensititre plate design, it was tested using a broth microtiter plate method, where 40 μl of antimicrobial was added to each well, 150 μl of 1.33 times concentration of Eaton's broth medium followed by 10 μl of inoculum. The plates were sealed with a plate sealer and incubated as described above.

Following incubation, the Sensititre and microtiter plates were centrifuged at 800 × *g* for 3 minutes to concentrate the cells. The plates were examined with an inverted mirror in a light box so that the growth or absence of growth of mycoplasma could be clearly observed, and the results were recorded. The end point was taken as the lowest dilution for which no buttons of cells could be seen.

The mycoplasmacidal (MMC) effect was also determined. After the MICs were read, 10 μl of culture was transferred into corresponding wells of another round-bottom microtiter plate containing 190 μl of Eaton's broth medium. This diluted the antimicrobials to below their effective inhibitory concentration so that the killing effect could be determined. The plates were incubated and examined as described above.

The MIC and MMC ranges and MIC_{50}, MIC_{90}, MMC_{50} (MMC at which 50% of mycoplasma isolates were killed), and MMC_{90} results are given in Table 1. All the duplicate results were within 1 dilution of each other, and in these cases, the highest concentration is given. Individual results for the most effective antimicrobials in vitro are given in Table 2, which excludes AMK, CEF, GEN, NAL, RIF, STR, TOB, and SXT.

TIL and OXYTET had the lowest MIC_{50} values of <0.06 μg/ml, followed closely by ERY and ENRO with values of <0.12 and 0.12 μg/ml, respectively. DAN, CIP, CLI, and LIN had values less than 1 μg/ml. TIL had the lowest MMC_{50} value at 0.25 μg/ml, followed by ENRO at 4.00 μg/ml. DAN, CIP, CLI, ERY, and LIN all had MMC_{50} values of 8.0 μg/ml.

In this study all eight isolates included from a previous study (1) gave comparable MIC results and were all within 2 dilutions of the results from the previous study. The MMC results were generally higher, which may relate to the unquantified number of cells used as inoculum in the MMC test; however, the effect of most antimicrobials is inhibitory rather than cidal.

The MIC range and the MIC_{50} values obtained for the recent African isolates and the other MmmSC isolates were comparable. TYL gave the widest range of MICs from 0.12 μg/ml to >64 μg/ml, indicating that some strains have developed resistance. While the MmmSC isolates tested in this study were limited to only a few countries, the MIC data obtained were similar, giving a useful baseline level for future MIC testing and monitoring for the development of antimicrobial resistance.

This study provides a basis for the selection of antimicrobials for use against MmmSC in vivo. While much debate will continue about the wisdom of using antimicrobials to treat CBPP, it has already been demonstrated in vivo that antimicrobials can prevent the spread of CBPP, even if they are not effective at curing the condition (4). Therefore, strategic antimicrobial treatment combined with other measures such as vaccination, movement control, and culling may help in the elimination of CBPP.

TABLE 2. Individual MIC and MMC values for *Mycoplasma mycoides* subsp. *mycoides* small-colony type isolates against 13 antimicrobials

Strain	Source (country, yr)	MIC (MMC) (μg/ml) of antimicrobial:												
		TIL	OXYTET	ERY	ENRO	CLI	DAN	CIP	LIN	CHL	FLO	NOR	SPT	TYL
V5	Australia, 1936	<0.06 (0.5)	0.12 (16)	0.25 (>32)	0.12 (4)	0.25 (8)	0.25 (4)	0.25 (8)	1 (32)	2 (32)	1 (16)	2 (32)	2 (64)	64
KH_3J	Sudan, 1940	<0.06 (0.5)	0.12 (16)	<0.12 (32)	0.25 (4)	0.25 (8)	0.25 (16)	0.5 (8)	0.5 (32)	2 (32)	2 (16)	8 (>32)	16 (>64)	>64
Afade	Chad, 1968	<0.06 (0.25)	0.12 (8)	<0.12 (>32)	0.25 (4)	0.25 (32)	0.25 (8)	0.25 (8)	1 (32)	2 (32)	1 (16)	2 (>32)	8 (>64)	16
2091	France, 1984	<0.06 (8)	<0.06 (4)	<0.12 (>32)	0.25 (8)	0.25 (32)	0.25 (8)	0.25 (8)	0.5 (>32)	1 (32)	1 (16)	2 (32)	16 (>64)	4
2022	France, 1984	<0.06 (0.5)	0.12 (8)	<0.12 (>32)	0.25 (4)	0.25 (8)	0.25 (8)	0.5 (8)	2 (32)	2 (32)	1 (16)	2 (32)	16 (>64)	64
138/5	Italy, 1992	<0.06 (2)	<0.06 (4)	<0.12 (>32)	0.25 (4)	0.25 (8)	0.25 (4)	0.25 (8)	2 (32)	2 (32)	1 (16)	2 (32)	16 (>64)	8
197	Italy, 1992	<0.06 (0.5)	<0.06 (8)	<0.12 (8)	0.12 (4)	<0.12 (8)	0.12 (8)	<0.12 (8)	<0.12 (32)	0.25 (32)	0.25 (16)	8 (>32)	4 (>64)	64
Madrid	Spain, 1993	<0.06 (0.25)	<0.06 (16)	0.25 (>32)	0.12 (4)	0.25 (8)	0.12 (8)	0.25 (8)	1 (32)	1 (32)	0.5 (16)	1 (32)	4 (>64)	16
B103	Portugal, 1986	<0.06 (0.5)	0.12 (4)	<0.12 (8)	0.12 (4)	<0.12 (32)	0.25 (8)	0.25 (8)	0.5 (32)	0.5 (32)	0.5 (16)	2 (>32)	8 (>64)	16
M545/91	Portugal, 1991	<0.06 (0.25)	0.12 (8)	<0.12 (8)	0.25 (4)	0.25 (8)	0.25 (8)	<0.12 (8)	0.5 (32)	1 (32)	1 (32)	1 (32)	4 (>64)	>64
B526	Portugal, 1993	<0.06 (1)	<0.06 (16)	<0.12 (>32)	0.25 (4)	<0.12 (32)	0.25 (16)	0.25 (8)	1 (>32)	1 (32)	2 (16)	2 (>32)	16 (>64)	16
6305	Portugal, 1993	<0.06 (0.5)	<0.06 (8)	<0.12 (8)	0.25 (8)	<0.12 (8)	0.25 (8)	0.25 (8)	0.25 (32)	1 (32)	0.5 (16)	2 (>32)	16 (>64)	16
410	Portugal, 1997	<0.06 (0.5)	<0.06 (4)	<0.12 (32)	0.25 (4)	0.25 (32)	0.25 (4)	0.25 (8)	0.5 (>32)	2 (32)	2 (16)	2 (>32)	16 (>64)	64
427	Portugal, 1997	<0.06 (0.25)	0.12 (16)	0.25 (8)	0.12 (8)	0.25 (8)	0.25 (8)	0.25 (8)	2 (>32)	2 (>32)	1 (32)	1 (>32)	4 (>64)	2
M375	Botswana, 1996	<0.06 (0.25)	0.12 (8)	0.25 (2)	0.12 (4)	0.25 (8)	0.12 (8)	0.25 (8)	0.5 (8)	0.25 (32)	0.5 (16)	2 (>32)	4 (>64)	8
N6	Botswana, 1996	<0.06 (0.25)	<0.06 (8)	<0.12 (2)	0.25 (4)	0.5 (2)	0.25 (16)	0.25 (8)	0.5 (8)	2 (32)	1 (16)	2 (32)	4 (>64)	0.25
TAN 8	Tanzania, 1996	<0.06 (0.25)	<0.06 (8)	<0.12 (1)	0.12 (2)	<0.12 (8)	0.25 (4)	0.25 (32)	0.5 (8)	1 (32)	0.5 (16)	2 (32)	4 (>64)	2
1S6	Tanzania, 1997	<0.06 (0.25)	<0.06 (4)	<0.12 (8)	0.12 (2)	<0.12 (2)	0.25 (8)	0.25 (8)	0.5 (8)	1 (32)	1 (16)	1 (32)	4 (>64)	16
1S18	Tanzania, 1997	<0.06 (0.25)	<0.06 (4)	<0.12 (2)	0.12 (2)	<0.12 (2)	0.25 (4)	0.25 (8)	0.5 (8)	0.5 (32)	1 (16)	2 (32)	8 (>64)	8
1S5	Tanzania, 1998	0.12 (0.5)	0.06 (64)	<0.12 (8)	0.25 (2)	0.5 (1)	0.25 (32)	<0.12 (8)	0.25 (>32)	1 (32)	1 (16)	1 (32)	4 (>64)	0.5
1S26	Tanzania, 1998	<0.06 (0.25)	0.12 (32)	<0.12 (8)	0.12 (4)	<0.12 (2)	0.25 (4)	0.25 (8)	0.5 (8)	1 (32)	2 (16)	2 (32)	8 (>64)	16
1S31	Tanzania, 1998	<0.06 (8)	0.12 (32)	<0.12 (2)	0.12 (2)	<0.12 (2)	0.12 (4)	<0.12 (8)	<0.12 (8)	1 (32)	1 (16)	2 (32)	4 (>64)	16
1S33	Tanzania, 1998	<0.06 (8)	<0.06 (8)	<0.12 (2)	0.12 (4)	<0.12 (2)	0.12 (4)	0.25 (32)	0.5 (8)	1 (>32)	0.5 (8)	1 (32)	8 (>64)	1
1S21	Tanzania, 1999	0.12 (8)	0.5 (32)	<0.12 (1)	0.12 (2)	<0.12 (2)	0.25 (32)	0.25 (8)	0.5 (32)	2 (32)	1 (16)	2 (32)	8 (>64)	64
1S22	Tanzania, 1999	<0.06 (0.25)	0.12 (16)	<0.12 (8)	0.12 (2)	<0.12 (2)	0.25 (32)	0.25 (8)	0.5 (32)	1 (32)	1 (16)	2 (32)	8 (>64)	64
1S23	Tanzania, 1999	<0.06 (0.25)	0.12 (16)	<0.12 (2)	0.5 (2)	<0.12 (8)	0.5 (32)	0.25 (8)	0.25 (32)	1 (32)	8 (>64)	2 (32)	16 (>64)	2
1S24	Tanzania, 1999	<0.06 (0.25)	0.12 (16)	<0.12 (2)	0.12 (4)	<0.12 (2)	0.12 (4)	0.25 (8)	0.25 (8)	0.5 (32)	1 (32)	2 (32)	4 (>64)	4
1S25	Tanzania, 1999	<0.06 (1)	0.12 (32)	<0.12 (>32)	0.12 (8)	<0.12 (2)	0.25 (8)	0.25 (8)	0.25 (8)	2 (32)	1 (16)	2 (32)	4 (>64)	2
1S27	Tanzania, 1999	0.12 (0.25)	0.25 (32)	<0.12 (8)	0.12 (4)	<0.12 (2)	0.25 (4)	0.25 (8)	0.5 (8)	2 (32)	1 (32)	2 (32)	8 (>64)	64
1S28	Tanzania, 1999	<0.06 (0.25)	0.25 (32)	0.25 (2)	0.12 (4)	0.25 (2)	0.25 (4)	0.25 (8)	1 (8)	2 (32)	2 (16)	2 (32)	8 (>64)	16
1S29	Tanzania, 1999	<0.06 (0.25)	0.12 (32)	<0.12 (2)	0.12 (2)	0.25 (2)	0.25 (4)	0.25 (8)	0.5 (32)	1 (32)	1 (16)	2 (32)	4 (>64)	16
1S30	Tanzania, 1999	<0.06 (16)	0.12 (64)	<0.12 (1)	0.12 (2)	<0.12 (2)	0.25 (8)	0.25 (8)	0.25 (8)	1 (>32)	0.5 (8)	2 (>32)	4 (>64)	16
1S32	Tanzania, 1999	<0.06 (0.25)	<0.06 (16)	<0.12 (2)	0.12 (2)	<0.12 (2)	0.12 (4)	0.25 (8)	0.25 (8)	0.5 (32)	0.5 (16)	2 (32)	4 (>64)	16
1S34	Tanzania, 1999	<0.06 (0.12)	<0.06 (32)	<0.12 (1)	0.12 (8)	<0.12 (2)	0.12 (4)	0.25 (8)	0.5 (8)	1 (32)	0.5 (16)	2 (32)	4 (>64)	16
1S35	Tanzania, 1999	<0.06 (0.25)	0.12 (32)	<0.12 (8)	0.12 (2)	<0.12 (2)	0.25 (16)	0.25 (8)	0.5 (8)	0.5 (32)	0.5 (16)	2 (32)	8 (>64)	16
26/3/01	Namibia, 2001	<0.06 (0.5)	<0.06 (4)	<0.12 (2)	0.25 (2)	0.5 (8)	0.25 (16)	0.5 (8)	1 (8)	2 (32)	1 (16)	2 (32)	8 (>64)	8
28/3/01	Namibia, 2001	<0.06 (0.25)	<0.06 (4)	<0.12 (2)	0.25 (2)	0.25 (8)	0.5 (16)	0.5 (8)	0.5 (8)	1 (32)	1 (16)	>32 (>32)	8 (>64)	2
SF0177	Namibia, 2004	<0.06 (0.25)	<0.06 (4)	<0.12 (8)	0.12 (4)	0.25 (8)	0.25 (8)	0.5 (8)	0.5 (32)	1 (32)	0.5 (16)	8 (>32)	8 (>64)	8
9809	Namibia, 2004	<0.06 (0.25)	<0.06 (4)	<0.12 (2)	0.25 (2)	<0.12 (2)	0.25 (32)	0.25 (8)	0.5 (8)	1 (32)	0.5 (16)	2 (>32)	8 (>64)	4
Okahao	Namibia, 2004	<0.06 (0.25)	<0.06 (4)	<0.12 (2)	0.25 (8)	0.25 (8)	0.25 (8)	0.25 (8)	0.5 (8)	1 (32)	0.5 (16)	2 (32)	4 (>64)	ND[a]
Matapi	Namibia, 2004	<0.06 (0.25)	<0.06 (4)	<0.12 (2)	0.25 (4)	0.25 (8)	0.25 (8)	0.25 (8)	0.25 (8)	1 (32)	0.5 (16)	2 (32)	4 (>64)	0.12

[a] ND, not determined.

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