In vitro antimicrobial susceptibility of bovine farcy organisms

M.E. Hamid [1*] M. Goodfellow [2]

Key words

Zebu cattle - *Mycobacterium farcinogenes* - *Mycobacterium senegalense* - *Nocardia farcinica* - Antibiotics - Resistance to chemicals - Antimicrobial - *In vitro* experiment - Sudan.

Summary

The *in vitro* activities of 35 various antimicrobial agents were tested against 42 *Mycobacterium farcinogenes*, 13 *M. senegalense*, 17 *Nocardia farcinica* strains using glucose yeast extract agar medium. Most of the *M. farcinogenes* strains were susceptible to amikacin (2 µg/ml), doxycycline HCl (8 µg/ml), erythromycin (64 µg/ml), lividomycin sulfate (16 µg/ml), minocycline HCl (8 µg/ml), nalidixic acid (64 µg/ml), oleandomycin phosphate (64 µg/ml), oxytetracycline HCl (64 µg/ml), rifampicin (64 µg/ml), streptomycin sulfate (64 µg/ml) and vancomycin (64 µg/ml). A distinct resistance profile to the antituberculous drugs was noted for most of the test strains, though *M. senegalense* and *N. farcinica* showed much more resistance than *M. farcinogenes*. The pattern of drug susceptibility to antimicrobial agents proved useful in differentiating between *M. farcinogenes* and *M. senegalense* and between these two and *N. farcinica*. It may have significant therapeutic implications in bovine farcy.

■ INTRODUCTION

Bovine farcy is a chronic infectious disease of zebu cattle that is characterized by granulomatous inflammation of the lymphatic system and subcutaneous tissues. The disease has long been recognized in many tropical countries and was thought to be caused by the actinomycetes, *Nocardia farcinica* (9, 13), but it is now known that *Mycobacterium farcinogenes* is the causal agent in Eastern and Central Africa and *M. senegalense* is the main agent in Western Africa (3). Yet, the role of *N. farcinica* in causing or being associated with diseases similar to bovine farcy cannot be ruled out since a report from Nigeria of cutaneous bovine lesions similar to bovine dermatophilosis caused by *N. farcinica* has been published (10).

Recently, *in vitro* susceptibility studies have revealed a number of antibacterial compounds against the rapidly growing mycobacteria (2, 8, 14) and against members of the genera *Gordona, Nocardia* and *Rhodococcus* (1, 15).

Although different methods have been used to assess the activity of selected antimicrobial agents against bovine farcy organisms (3, 4, 12), little is known of the activities of the commonly used as well as the new antibacterial compounds against organisms causing bovine farcy.

The aim of the present study was to determine the *in vitro* susceptibility of various antimicrobial agents representing a wide range of structural types and modes of action against isolates of *M. farcinogenes*, *M. senegalense* and *N. farcinica*. The data obtained may provide basic information towards establishing a line of treatment in animals infected with bovine farcy. The disease is still causing a considerable economic loss in many African countries, notably the Sudan (7).

■ MATERIALS AND METHODS

The test strains consisting of 42 *M. farcinogenes*, 13 *M. senegalense*, 17 *Nocardia farcinica* strains were cultivated on glucose yeast extract agar (GYEA) (5) for 7 to 14 days at 37°C, and then checked for purity prior to use. A cellular suspension of each of the test strains was prepared in Universal bottles containing 1/4 strength Ringer solution (Oxoid). Detailed information on the source and strain history have been recorded earlier (6).

The test strains were examined for their ability to grow in GYEA in the presence of each of 35 antibiotics and antibacterial agents at various concentrations (table I). All but one of the antibiotics were sterilized by filtering aqueous solutions, at suitable concentrations, through 0.22 µm Millipore filters (Millipore Corp., Bedford, Massachusetts, USA) into Universal bottles. The remaining antibiotics, rifampicin, was dissolved in dimethylformamide containing an equal volume of ethanol prior to sterilization by filtering as above. Sterilized solutions of each antibacterial agent were added to cooled molten GYEA to give an appropriate concentration. Media were dispensed into Petri dishes and inoculated immediately after they had solidified.

1. Department of Medicine, Pharmacology and Toxicology, Faculty of Veterinary Science, University of Khartoum, Khartoum North, PO Box 32, Sudan

2. Department of Microbiology, the Medical School, University of Newcastle, Newcastle upon Tyne, NE2 4HH, United Kingdom

* Corresponding author

Table I

Percentages of resistant strains of *Mycobacterium farcinogenes*, *M. senegalense* and *Nocardia farcinica* to some antimicrobial agents

Antimicrobial agents (µg/ml)	*M. farcinogenes* (n = 42 strains) % of strains	*M. senegalense* (n = 13 strains) % of strains	*N. farcinica* (n = 17 strains) % of strains
Aminoglycosides			
Amikacin (2)	7	17	100
Amikacin (16)	7	12	100
Bekanamycin sulfate (2)	100	100	100
Bekanamycin sulfate (16)	49	50	88
Capreomycin sulfate (10)	20	17	100
Capreomycin sulfate (40)	2	17	82
Capreomycin sulfate (160)	0	8	76
Gentamycin sulfate (32)	100	33	100
Gentamycin sulfate (64)	32	17	100
Gentamycin sulfate (128)	0	0	0
Lividomycin sulfate (2)	98	100	100
Lividomycin sulfate (16)	5	25	88
Neomycin sulfate (64)	95	92	100
Neomycin sulfate (128)	12	25	100
Parmomycin sulfate (64)	12	17	100
Streptomycin sulfate (16)	100	100	100
Streptomycin sulfate (32)	80	75	100
Streptomycin sulfate (64)	9	33	41
Antituberculous compounds *			
p-Aminosalicylic acid, Na salt (162)	98	100	100
p-Aminosalicylic acid, Na salt (64)	22	100	100
D-cycloserine (2)	98	100	100
D-cycloserine (64)	98	100	100
D-cycloserine (128)	95	100	100
Ethambutol HCl (4)	100	100	100
Ethambutol HCl (32)	98	83	100
Ethambutol HCl (64)	66	92	12
Ethionamide (5)	100	83	100
Ethionamide (50)	12	83	100
Isoniazid (2)	100	100	100
Isoniazid (16)	100	92	100
Isoniazid (64)	98	92	100
Isoniazid (128)	12	75	100
Thiacetazone (10)	100	58	100
Cephalosporins			
Cephaloridine (64)	68	92	59
Cephalopirin Na salt (64)	93	100	82
Cephalopirin Na salt (128)	15	100	73
Cumarin			
Novobiocin (64)	63	100	100
Lincosamides			
Lincomycin HCl (64)	95	83	100
Lincomycin HCl (128)	98	100	100
Macrolides			
Erythromycin (64)	5	92	100
Erythromycin (128)	0	92	100
Oleandomycin phosphate (64)	12	100	100
Peptides			
Polymyxin B sulfate (64)	7	75	35
Vancomycin HCl (64)	5	8	47
Viomycin sulfate (64)	0	17	100

* Some antituberculous drugs (e.g. streptomycin, rifampicin) have been classified under other structural headings

Table I (continued)

Antimicrobial agents (µg/ml)	*M. farcinogenes* (n = 42 strains) % of strains	*M. senegalense* (n = 13 strains) % of strains	*N. farcinica* (n = 17 strains) % of strains
Penicillins (ß-lactam)			
Amoxicillin (64)	93	100	100
Amoxicillin (128)	5	92	59
Ampicillin (64)	63	100	100
Penicillin G, K salt (66 IU/ml)	54	100	100
Quinolone			
Nalidixic acid, Na salt (64)	7	83	53
Rifampicin			
Rifampicin (16)	100	92	100
Rifampicin (64)	12	75	94
Sulphonamides			
Dapsone (16)	95	100	100
Dapsone (64)	98	100	100
Sulphamethazine (16)	100	100	100
Sulphamethazine (64)	98	100	100
Sulphamethazine (128)	56	92	0
Trimethoprim+sulphamethoxazole (8)	98	100	100
Trimethoprim+sulphamethoxazole (64)	98	92	100
Tetracyclines			
Chlortetracycline HCl (64)	24	33	88
Doxycycline HCl (8)	5	67	88
Doxycycline HCl (32)	0	17	12
Doxycycline HCl (64)	0	8	0
Minocycline HCl (64)	10	0	0
Oxytetracycline HCl (64)	2	8	76

Plates were inoculated from 1/4 strength Ringer's suspension. Inoculation was carried out using a multipoint inoculator (Henley-Tech, Henley Instrument Ltd., Billinghurst, England) and a procedure that allowed the standardization and multiple surface inoculation of 90 mm diameter Petri dishes. The multipoint inoculator was fitted with an inoculation head that carried 20 pins for culture transfer and one marked pin as a reference point to orientate plates. Inocula were pipetted into sterile, inverted Oxoid caps held in an array within a metal template placed inside the base of a 100 mm square Replidish (Sterilin, Teddington, England).

Inoculated plates were incubated at 37˚C for 7 days prior to visual inspection. Growth of the culture in the presence of the antibacterial agents was compared to that on the GYEA control plate. A positive result was noted when growth was recorded, i.e. when the organisms showed no resistance to the antibacterial compounds. Members of the *M. farcinogenes* which showed no growth after 7 days were incubated 7 more days and examined as above, because these organisms grow relatively slower than *M. senegalense* and *N. farcinica*.

■ RESULTS AND DISCUSSION

The results of the *in vitro* antimicrobial sensitivity testing are presented in table I. All strains of *M. farcinogenes* were susceptible to doxycycline HCl (32 µg/ml), capreomycin sulfate (160 µg/ml), erythromycin (128 µg/ml), gentamycin sulfate (128 µg/ml) and viomycin sulfate (64 µg/ml). Most of the strains were susceptible to amikacin (2 µg/ml), capreomycin sulfate (40 µg/ml), cephapirin Na salt (128 µg/ml), erythromycin (64 µg/ml), ethionamide (50 µg/ml), lividomycin sulfate (16 µg/ml), minocycline HCl (64 µg/ml), nalidixic acid Na salt (64 µg/ml), oleandomycin phosphate (64 µg/ml), oxytetracycline HCl (64 µg/ml), polymyxin B sulfate (64 µg/ml), rifampicin (64 µg/ml), streptomycin sulfate (64 µg/ml) and vancomycin HC1 (64 µg/ml). *M. senegalense* and *N. farcinica* strains were found to be more resistant to the antimicrobial agents tested than *M. farcinogenes*. However, the growth of *M. senegalense* strains was inhibited by gentamycin sulfate (128 µg/ml) and minocycline HCl (64 µg/ml) and that of *N. farcinica* strains by gentamycin sulfate (128 µg/ml), sulphamethazine (128 µg/ml), doxycycline HCl (64 µg/ml) and minocycline HCl (64 µg/ml).

A distinct resistance profile to the antituberculous drugs (figure 1), including *p*-aminosalicylic acid (16 µg/ml), bekanamycin sulfate (2 µg/ml), D-cycloserine (64 µg/ml), dapsone (16 µg/ml), ethambutol (32 µg/ml), ethionamide (5 µg/ml), isoniazid (64 µg/ml), rifampicin (16 µg/ml), streptomycin sulfate (32 µg/ml), and thiacetazone (10 µg/ml), was noted for most of the *M. farcinogenes*, *M. senegalense* and *N. farcinica* strains. However, at relatively high MICs most of the *M. farcinogenes* strains were susceptible to capreomycin sulfate (40 µg/ml), ethionamide (50 µg/ml), isoniazid (128 µg/ml), rifampicin (64 µg/ml) and streptomycin sulfate (32 µg/ml) (figure 1).

Treatment with multiple agents is preferable because of the high relapse rates and emergence of drug resistance. Resistance to antibiotics, notably the plasmid mediated resistance, has been studied intensively in many mycobacteria species. Wallace *et al.* (14) have demonstrated that the resistance of the rapidly growing mycobacteria such as *M. fortuitum* and *M. chelonae* to single-drug therapy could be attributed to mutational resistance. The rapidly growing mycobacteria, including *M. farcinogenes* and

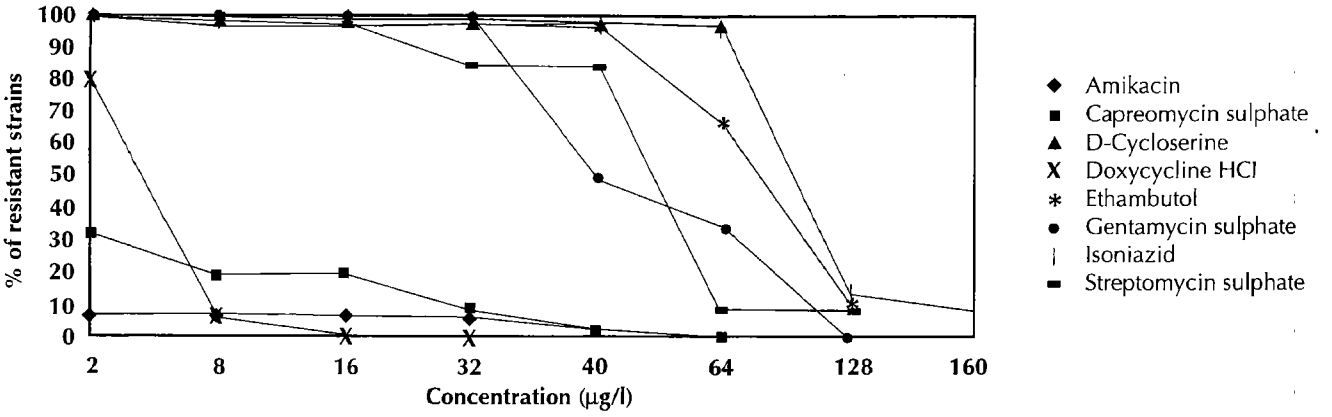

Figure 1: *resistance patterns of* Mycobacterium farcinogenes *strains to selected antibacterial agents.*

Table II

Antimicrobial agents with greatest resolution for differentiating between *Mycobacterium farcinogenes, M. senegalense* and *Nocardia farcinica* strains

Antimicrobial agents (µg/ml)	*M. farcinogenes*	*M. senegalense*	*N. farcinica*
Amikacin (2)	-	-	+
Amoxicillin (128)	-	+	+/-
Capreomycin sulfate (10)	-/+	-	+
Capreomycin sulfate (40)	-	-	+
Cephalopirin Na salt (128)	-	+	+/-
Doxycycline HCl (8)	-	+/-	+
Erythromycin (64)	-	+	+
Ethambutol HCl (64)	+/-	+	+
Ethionamide (50)	-	+	+
Gentamycin sulfate (64)	-/+	-	+
Isoniazid (128)	-	+/-	+
Lividomycin sulfate (16)	-	-/+	+
Nalidixic acid, Na salt (64)	-	+	+/-
Neomycin sulfate (128)	-	-/+	+
Oleandomycin phosphate (64)	-	+	+
Parmomycin sulfate (64)	-	-	+
Rifampicin (64)	-	+/-	+
Streptomycin sulfate (32)	-	-/+	+
Sulphamethazine (128)	+	+	-
Viomycin sulfate (64)	-	-	+

+ means more than 80% of strains positive; - means more than 80% negative; -/+ means 20 to 49% positive; +/- means 50 to 79% of the strains positive.

M. senegalense, vary greatly in their *in vitro* susceptibility to the currently used antibacterial agents, as recorded in the present investigation, and in a agreement with other authors' work (2, 8, 14). Using data derived from the analysis of the 16S rRNA, Pitulle *et al.* (11) accommodated both *M. farcinogenes* and *M. senegalense* in the phylogenetic branch of the rapidly growing species of mycobacteria and found they were closely related to *M. chelonae* and *M. fortuitum*, non-photochromogenic mycobacteria and opportunistic human pathogens.

The pattern of drug susceptibility to antimicrobial agents is found to be advantageous in differentiating between *M. farcinogenes* and *M. senegalense* and between these two and *N. farcinica* (table II). *M. farcinogenes* and *M. senegalense*, but not *N. farcinica*, were sensitive to amikacin (2 µg/ml), parmomycin sulfate (64 µg/ml) and capreomycin sulfate (40 µg/ml). *M. farcinogenes*, but not *M. senegalense* and *N. farcinica*, was resistant to ethambutol (50 µg/ml), erythromycin (16 µg/ml) and oleandomycin phosphate (64 µg/ml). The findings of the present study may have an important therapeutic impact on bovine farcy and its *in vitro* application. The data presented here indicate that *M. farcinogenes*, the main cause of bovine farcy in the Sudan, is susceptible to many agents such as capreomycin, doxycycline, erythromycin, gentamycin and viomycin at relatively high MICs. It is interesting that most of the strains of *M. farcinogenes* are susceptible to amikacin at MIC of 2 µg/ml and doxycycline at 8 µg/ml, which seems to be, when administered individually or in combination with other agents, suitable choices for a long lasting problem. The inconvenience usually arises when handling infected cattle belonging to nomads, whose lifestyle and continuous movements make frequent and suitable follow-up rather an arduous task.

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REFERENCES

1. BOIRON P., PROVOST F., 1990. Characterization of *Nocardia, Rhodococcus* and *Gordona* species by *in vitro* susceptibility testing. *Int. J. Med. Microbiol.*, **274**: 203-213.

2. BROWN B.A., WALLACE R.J. Jr., ONYI G.O., DE ROSAS V., WALLACE R.J., 1992. Activities of four macrolides, including clarithromycin, against *Mycobacterium fortuitum, Mycobacterium chelonae*, and *Mycobacterium chelonae*-like organisms. *Antimicrob. Agents Chemoth.*, **36**: 180-184.

3. CHAMOISEAU G., 1979. Aetiology of farcy in African bovines: nomenclature of the causal organisms *Mycobacterium farcinogenes* (Chamoiseau) and *Mycobacterium senegalense* (Chamoiseau) comb. nov. *Int. J. Syst. Bacteriol.*, **29**: 407-410.

4. EL SANOUSI S.M., SALIH M.A.M., MOUSA M.T., TAG EL DIN M.H., ALI S.A.M., 1979. Further studies on the properties of the aetiology of bovine farcy isolated from Sudanese cattle. *Revue Elev. Méd. vét. Pays trop.,* **32**: 135-141.

5. GORDON R.E., MIHM J.M., 1962. Identification of *Nocardia caviae* (Erikson) nov. comb. *Ann. N.Y. Acad. Sci.,* **98**: 628-636.

6. HAMID M.E., MINNIKEN D.E., GOODFELLOW M., 1993. A simple chemical test to distinguish mycobacteria from other mycolic acid containing actinomycetes. *J. Gen. Microbiol.*, **139**: 2203-2213.

7. HAMID M.E., MOHAMED G.E., ABU-SAMRA M.T., El SANOUSI S.M., BARRI M.E., 1991. Bovine farcy: a clinico-pathological study of the disease and its aetiological agent. *J. Comp. Pathol.,* **105**: 287-301.

8. HOEL T., CASALS J.B., ENG J., 1993. *In vitro* antimicrobial susceptibility testing of rapidly growing mycobacteria using the tablet diffusion method: resistance pattern of Norwegian *Mycobacterium fortuitum* and *Mycobacterium chelonae* isolates. *APMIS*, **101**: 27-32.

9. MOSTAFA I.E., 1966. Bovine nocardiosis (cattle farcy): a review. *Vet. Bull.*, **36**: 189-193.

10. OYEKUNLE M.A., OJO M.O., 1988. Preliminary obervations on bovine cutaneous nocardiosis and dermatophilosis in the subhumid climate of Southern Nigeria. *Revue Elev. Méd. vét. Pays trop.,* **41**: 347-351.

11. PITULLE C., DORSCH M., KAZDA J., WOLTERS J., STACKEBRANDT E., 1992. Phylogeny of rapidly growing members of the genus *Mycobacterium. Int. J. Syst. Bacteriol.*, **42**: 337-343.

12. RIDELL M., GOODFELLOW M., ABDULLE M.A., 1985. Identification of actinomycetes isolated from cases of bovine farcy in the Sudan. *Zbl. Bakt. Mikrobiol. Hyg.*, **A259**: 43-50.

13. TREVISAN V., 1952. In: *Int. Bull. Bacteriol. Nomencl. Tax.*, **2**. Quoted from: I Generi e le Specie delle Batteriacaee, 1889. Milano, Italia, Zanaboni e Gabuzzi.

14. WALLACE R.J. Jr., BEDSOLE G., SUMTER G., SANDERS C.V., STEELE L.C., BROWN B.A., SMITH J., GRAHAM D.R., 1990. Activities of ciprofloxin and ofloxacin against rapidly growing mycobacteria with demonstration of acquired resistance following single-drug therapy. *Antimicrob. Agents Chemoth.*, **34**: 65-70.

15. WALLACE R.J. Jr., TSUKAMURA M., BROWN B.A., BROWN J.M., STEINGRUDE V.A., ZHANG Y., NASH D.R., 1990. Cefotaxime resistant *Nocardia asteroides* strains are isolates of the controversial species *Nocardia farcinica. J. clin. Microbiol.,* **28**: 2726-2732.

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Résumé

Hamid M.E., Goodfellow M. Test *in vitro* de la sensibilité des agents du farcin à différents agents antimicrobiens

Trente-cinq agents antimicrobiens différents ont été testés *in vitro* sur 42 souches de *Mycobacterium farcinogenes*, 13 de *Mycobacterium senegalense* et 17 de *Nocardia farcinica* sur un milieu solide avec extrait de levure glucosé. La plupart des souches étaient sensibles à l'amikacine (2 µg/ml), à la doxycycline (8 µg/ml), à l'érythromycine (64 µg/ml), au sulfate de lividomycine (16 µg/ml), à la minocycline (8 µg/ml), à l'acide nalidixique (64 µg/ml), au phosphate d'oléandomycine (64 µg/ml), à l'oxytétracycline (64 µg/ml), à la rifampicine (64 µg/ml), au sulfate de streptomycine (64 µg/ml) et à la vancomycine (64 µg/ml). Une nette résistance aux médicaments antituberculeux a été observée dans la plupart des souches testées, bien que *M. senegalense* et *N. farcinica* aient montré beaucoup plus de résistance que *M. farcinogenes*. Les différentes réponses des médicaments aux agents antimicrobiens se sont avérées utiles pour différencier *M. farcinogenes* et *M. Senegalense*, ainsi que ces deux derniers et *N. farcinica*. Ceci pourrait avoir des conséquences thérapeutiques significatives dans le traitement du farcin.

Mots-clés : Bovin - Zébu - *Mycobacterium farcinogenes* - *Mycobacterium senegalense* - *Nocardia farcinica* - Antibiotique - Résistance aux produits chimiques - Antimicrobien - Expérimentation *in vitro* - Soudan.

Resumen

Hamid M.E., Goodfellow M. Susceptibilidad anti microbiana *in vitro* de los organismos granulomatosos bovinos

Se examinó la actividad *in vitro* de 35 agentes antimicrobianos contra 42 cepas de *Mycobacterium farcinogenes*, 13 *M. senegalense*, 17 *Nocardia farcinica*, mediante el uso de un medio agar de extracto de levadura y glucosa. La mayoría de las cepas de *M. farcinogenes* fueron susceptibles a la amicacina (2 µg/ml), HCl de doxiciclina (8 µg/ml), eritromicina (64 µg/ml), sulfato de lividomicina (16 µg/ml), HCl de minociclina (8 µg/ml), ácido nalidíxico (64 µg/ml), fosfato de oleandomicina (64 µg/ml), HCl de oxitetraciclina (64 µg/ml), rifampicina (64 µg/ml), sulfato de estreptomicina (64 µg/ml) y vancomicina (64 µg/ml). En la mayor parte de las cepas se observó un comportamiento de resistencia diferente a las drogas antituberculosas, a pesar de que *M. senegalense* y *N. farcinica* mostraron mucha más resistencia que *M. farcinogenes*. El patrón de susceptibilidad a los agentes antimicrobianos fue importante en la diferenciación entre *M. farcinogenes* y *M. senegalense*, así como entre estos dos y *N. farcinica*. Podría tambien conllevar importantes implicaciones terapeúticas contra las enfermedades granulomatosas en el bovino.

Palabras clave: Ganado bovino - Cebú - *Mycobacterium farcinogenes* - *Mycobacterium senegalense* - *Nocardia farcinica* - Antibiótico - Resistencia a productos químicos - Antimicrobiano - Experimentación *in vitro* - Sudan.