

In Vitro Antimicrobial Susceptibility Testing of Animal *Nocardia* Isolated from Field Cases of Skin Diseases

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Key words

Ruminant – Domestic animal –
Nocardia sp. – Antimicrobial –
Resistance to chemicals – Nigeria.

Summary

In vitro antimicrobial tests were carried out on strains of *Nocardia* isolated from field cases of cutaneous nocardiosis in farm animals. Results with the disc diffusion test showed the multiresistant nature of the isolates, but 23.81 and 21.43% were sensitive to ciprofloxacin and gentamycin, respectively. The MIC mode and range for oxytetracycline were 12.5 and 3.12–25 µg/ml, respectively, while those of erythromycin were 3.12 and 0.78–6.25 µg/ml, respectively.

■ INTRODUCTION

Nocardiosis caused by *Nocardia* sp. is a disease affecting a wide range of domestic animals and man (5) and occurs principally in the tropical countries. The genera most easily confused with *Nocardia* are *Mycobacterium*, *Rhodococcus* and *Corynebacterium*, all of which contain mycolic acid and shared cell-wall type IV of meso-diaminopimelic acid with arabinose and galactose (6). Thus, recognition of the infection caused by *Nocardia* sp. depends on the laboratory ability to identify this species.

Nocardiosis in Nigeria occurs in two forms: (i) the cutaneous form, which resembles dermatophilosis (10), and (ii) the pulmonary form, which resembles tuberculosis (1). The resemblance of cutaneous nocardiosis and dermatophilosis has been responsible for the paucity of information on the incidence of the latter disease in Nigeria (7, 8). It is also one of the reasons given to account for the therapy resistance outbreaks of bovine dermatitis being

reported from the field because therapy meant for dermatophilosis is being applied for cutaneous nocardiosis (10).

In cattle, economic losses due to cutaneous nocardiosis and dermatophilosis can be through hide damage, impaired growth, lowered production and death because of debility (9). Therefore, rearing of cattle in Nigeria requires prophylactic and therapeutic measures to control these diseases.

This study attempted to determine the appropriate antimicrobial(s) against the causative agent of cutaneous nocardiosis in Nigeria.

■ MATERIALS AND METHODS

Isolates

Forty-two isolates of *Nocardia* sp. comprising 39 strains from cattle, 2 strains from sheep and 1 strain from goat (Table I) were used for the *in vitro* antimicrobial susceptibility study. The isolates were obtained from scabs or scrapings aseptically collected from infected skin sites of the affected animals at various farms in Southwestern Nigeria. Primary isolation of the organisms was carried out on brain heart infusion agar (Oxoid) as previously described (11). The isolates were identified as *Nocardia* sp. according to the methods described by Lechevalier and Lechevalier (6) and Holt *et al.* (4).

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Table I

Zones of inhibition produced by the antimicrobials to the test strains of *Nocardia* sp.

Bacteria Strain*	Zones of inhibition (mm)							
	Ampicillin (Amp 10 µg)	Amoxycillin (Am 20 µg)	Gentamycin (Gen 10 µg)	Cephalexin (Cx 10 µg)	Cloxacillin (OB 10 µg)	Ciprofloxacin (Cip 5 µg)	Erythromycin (E 15 µg)	Streptomycin (S 5 µg)
1	0	0	15	0	0	16	0	10
2	0	0	10	0	0	12	10	15
3	0	0	0	0	0	5	0	0
4	0	0	10	0	0	15	0	5
5	0	0	10	0	0	15	0	0
6	0	0	3	0	0	0	0	0
7	0	0	5	0	0	10	0	0
8	0	0	15	0	0	6	0	0
9	0	0	10	0	0	0	0	9
10	0	0	12	0	15	15	0	0
11	0	0	0	0	0	15	5	0
12	0	0	15	0	0	10	0	6
13	0	0	8	0	0	5	0	10
14	0	0	16	0	0	5	0	0
15	0	0	15	0	0	10	0	0
16	0	0	10	0	0	14	0	0
17	0	0	5	0	0	10	15	0
18	0	0	3	0	0	15	0	15
19	0	0	10	0	0	5	10	10
20	0	0	15	8	0	5	0	0
21	0	3	8	0	0	0	0	5
22	0	0	15	0	0	10	0	10
23	0	0	10	0	0	12	0	0
24	0	0	10	0	0	10	0	0
25	0	0	10	0	0	15	3	0
26	0	0	5	0	0	10	0	0
27	0	0	15	5	0	0	0	0
28	0	0	0	0	0	5	0	5
29	0	0	16	0	0	10	8	0
30	0	0	10	0	3	12	0	12
31	0	0	5	0	0	10	10	5
32	0	0	10	0	0	15	0	0
33	0	0	15	3	0	15	5	0
34	5	0	3	0	3	10	0	0
35	0	0	15	0	0	6	0	0
36	0	0	8	0	0	0	0	16
37	0	0	10	0	0	5	0	0
38	0	0	5	3	0	10	12	0
39	0	0	10	0	0	8	0	0
40	0	0	10	0	0	10	16	8
41	0	0	5	5	0	5	0	0
42	0	0	10	0	0	15	0	5

* 1-39: bovine strains; 40 and 41: ovine strains; 42: caprine strain

Antimicrobial disc susceptibility test

A 4.0-ml brain heart infusion broth of *Nocardia* sp. incubated at 37°C for 48 h and standardized to contain 3.14×10^4 cfu/ml of the organism was seeded onto brain heart infusion agar plate with a sterile cotton swab. The plates were inverted to dry for 15 min. Multidisc (Broadisk No. 2020p, Bronila) was placed on the surface of each of the inoculated agar plate with sterile forceps. The plates were then incubated under carbon dioxide enriched atmosphere at 37°C for 72 h (11). A zone of inhibition of 14 mm or less was regarded as resistance to the tested antimicrobials (2).

Determination of the minimum inhibitory concentration of oxytetracycline and erythromycin for *Nocardia* sp.

The minimum inhibitory concentrations (MIC) of oxytetracycline and erythromycin (Sigma Chemical) were determined by doubling dilution of the antimicrobials in the following concentration range: oxytetracycline, 0.25–100 µg/ml; and erythromycin, 0.39–12.5 µg/ml (final concentrations in brain heart infusion agar). A 48-hour brain heart infusion broth culture of the bacteria standardized to contain 3.14×10^4 cfu/ml was used as inoculum to seed the antimicrobial plates. The plates were then incubated at 37°C for 72 h under carbondioxide-enriched atmosphere (11). Oxford *Staphylococcus* NCTC 6571 was used as positive control. MIC was the lowest concentration of the antimicrobial that prevented the growth of the bacteria after 72-hour incubation.

RESULTS

Table I shows the zones of inhibition produced by the antimicrobials to the test strains of *Nocardia* sp. Table II shows the *in vitro* antimicrobial susceptibility pattern of the isolates of *Nocardia* sp. All the tested strains were resistant to ampicillin, amoxycillin and cephalixin. Ten strains (23.81%) were sensitive to gentamycin, one strain (2.38%) to cloxacillin and nine strains (21.43%) to ciprofloxacin. All these were of bovine origin (Table I). Two strains (4.76%), one bovine and one ovine, were sensitive to erythromycin (Table I). The three strains that were sensitive to streptomycin were of bovine origin.

The MIC mode and range for oxytetracycline were 12.5 and 3.12–25 µg/ml, respectively, while those of erythromycin were 3.12 and 0.78–6.25 µg/ml, respectively (Table II).

DISCUSSION

The result of the antimicrobial disc susceptibility test in this study showed that strains of *Nocardia* sp. examined were multiresistant (Tables I and II). The isolates were resistant to most antimicrobials used in veterinary practice in Nigeria. The indications for the use of some of these drugs are wide and their indiscriminate use may be partly responsible for the observed resistance to them by the tested strains. Although some of the tested strains were sensitive to ciprofloxacin and gentamycin (Table II), these drugs were not readily available in Nigeria for veterinary application.

With the conventional recommended doses, the effective plasma concentration of erythromycin and oxytetracycline are 0.5–2 and 0.1–10 µg/ml, respectively (3). These values were lower than the MIC obtained for each of the drugs (Table II). It was to be expected from these MIC values that a relatively high plasma concentration of the antimicrobial would be required to effect cure. While the MIC values obtained for oxytetracycline showed the drug to be moderately effective (Table II), previous reports suggest that it is the drug of choice in the treatment of cutaneous nocardiosis in Nigeria (10).

On the other hand, the value of antimicrobial susceptibility test of *Nocardia* sp. goes beyond what can be achieved in the chemotherapy of field cases (12, 13). Wallace *et al.* were able to separate *Nocardia asteroides*, *N. farcinica* and *N. nova* based on their antimicrobial susceptibility patterns (12, 13).

CONCLUSION

There was evidence from the results (Tables I and II) to suggest that the tested organisms were multiresistant. The problem of adequate therapy of cases can be resolved by conducting randomized field trials for the treatment of animals with cutaneous nocardiosis using some of the antimicrobials found to be effective in this study.

Table II

In vitro susceptibility pattern of *Nocardia* sp. to antimicrobials

Antimicrobial agents	Num. sensitive	%	Num. resistant	%	MIC (µg/ml)	
					mode	range
Ampicillin (10 µg)	0	0	42	100		
Amoxycillin (20 µg)	0	0	42	100		
Gentamycin (10 µg)	10	23.81	32	76.19		
Cephalexin (10 µg)	0	0	42	100		
Cloxacillin (10 µg)	1	2.38	41	97.62		
Ciprofloxacin (5 µg)	9	21.43	33	78.57		
Streptomycin (5 µg)	3	7.14	39	92.86		
Erythromycin (15 µg)	2	4.76	40	95.25	3.12	0.78–6.25
Oxytetracycline	-	-	-	-	12.5	3.12–25

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Résumé

Oyekunle M.A., Adetosoye A.I. Tests de sensibilité antimicrobienne *in vitro* de *Nocardia* isolée sur le terrain chez des animaux affectés d'une dermatose

Des tests antimicrobiens *in vitro* ont été effectués sur des souches de *Nocardia* isolée sur le terrain dans des cas de nocardiose chez des animaux d'élevage. Les résultats obtenus par la méthode des disques ont montré la nature multirésistante des isolats, bien que 23,81 et 21,43 p. 100 d'entre eux aient été sensibles respectivement à la ciprofloxacine et à la gentamicine. Les valeurs des concentrations minimales inhibitrices et leurs étendues ont été respectivement de 12,5 et 3,12-25 µg/ml pour l'oxytétracycline, et de 3,12 et 0,78-6,25 µg/ml pour l'érythromycine.

Mots-clés : Ruminant – Animal domestique – *Nocardia* sp. – Antimicrobien – Résistance aux produits chimiques – Nigeria.

Resumen

Oyekunle M.A., Adetosoye A.I. Pruebas de susceptibilidad anti microbiana *in vitro* de *Nocardia* animal aislada de casos de campo en enfermedades de la piel

Se llevaron a cabo pruebas anti microbianas *in vitro* en cepas de *Nocardia* aislada a partir de casos de campo de nocardiosis cutánea en animales de finca. Los resultados de la prueba de difusión con disco mostraron la naturaleza multi resistente de los aislamientos, sin embargo, 23,81 y 21,43% fueron sensibles a la ciprofloxacina y gentamicina, respectivamente. La moda y el rango para el MIC de la oxitetraciclina fue de 12,5 y 3,12-25 µg/ml, respectivamente, mientras que para la eritromicina fueron de 3,12 y 0,78-6,25 µg/ml, respectivamente.

Palabras clave: Rumiante – Animal doméstico – *Nocardia* sp. – Antimicrobiano – Resistencia a productos químicos – Nigeria.