In Vitro Antimicrobial Sensitivity Testing of *Nocardia Africana* Strains Recently Isolated from Patients with Pulmonary Infections in Sudan

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Objective: The aim of the present study was to determine the *in vitro* susceptibility of *Nocardia africana* to various antimicrobial agents. *Nocardia africana* is a Gram-positive aerobic actinomycete and a notable pulmonary pathogen.

Methods: *N. africana* strains were tested for their *in vitro* sensitivity against 34 different antimicrobial agents. The antimicrobial susceptibility was determined by the disk diffusion method using Mueller-Hinton agar medium. The zone of inhibition was read after 36-48 h of incubation at 37 degrees C.

Results: The results indicated that all *N. africana* isolates were sensitive to ciprofloxacin (5μg/ml), clindamycin (10μg/ml), fusidic acid (10μg/ml), gentamycin (10μg/ml), imipenem (10μg/ml), tobramycin (10μg/ml), amikacin (20μg/ml), doxycycline (30 μg/ml), minocycline (30μg/ml), and vancomycine (30μg/ml). They were resistant to compound sulfonamides (300μg/ml), sulphafurazole (100μg/ml), metronidazole (50μg/ml), aztereonam (30μg/ml), cefotetan (30μg/ml) nalidixic acid (30μg/ml) and penicillin G (10 units).

Conclusions: *N. africana* revealed distinct susceptibility and resistance profiles to antimicrobial agents testes. The study underline the importance of antimicrobial susceptibility testing for clinical isolates of *Nocardia* spp. Individual species show considerable differences in their susceptibility patterns which necessitate therapeutic adjustments and early prompt medical intervention.

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Nocardiae are Gram positive aerobic actinomycetes, and are known to cause a variety of localized and disseminated suppurative infections in humans and animals[1,2]. The most commonly reported pathogenic species are *Nocardia asteroides, N. farcinica* and *N. nova*, followed in importance by *N. brasiliensis, N. otididiscaviarum, N. pseudobrasiliensis* and *N. transvalensis*. Infections caused by *Nocardia* species are infrequent but challenging to clinicians. In recent years, the number of case reports has been increasing, and this can be attributed to the improvements in diagnostic capabilities and the higher clinical index of suspicion accompanying the increased prevalence of immunosuppressed patients[3,4]. Although nocardiosis had been reported from most regions of the world, but it is well established that nocardiae can be easily

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overlooked under routine culture and smear examinations. Moreover its incidence in many countries remains unknown.

In vitro susceptibility studies have revealed a number of antibacterial compounds found to be effective against different species of *Nocardia*. Wallace and Steele recommended routine susceptibility testing of *Nocardia* because not all patients are able to tolerate or show a favorable response to sulfonamides, the treatment of choice for *Nocardia*[5]. Many new drugs are available with good activity against nocardiae particularly *N. asteroides*, the most common pathogenic species. Susceptibility to agents including amikacin, amoxicillin/clavulanic acid, and the third generation cephalosporins has beenvariable[6-9].

The aim of the present study was to determine the *in vitro* susceptibility of various antimicrobial agents representing a wide range of structural type and modes of action against representatives isolates of *Nocardia africana* and in comparison to *N. asteroides* and *N. farcinica.* The data obtained may provide basic information towards establishing a line of treatment in humans infected with chronic lung disease caused by nocardiae notably *N. africana. N. africana* has recently been found to cause a significant number of pulmonary infections among Sudanese patients attending respiratory disease clinics with symptoms simulating tuberculosis[10].

METHODS

Strains and culture conditions

Six *Nocardia africana* strains: SD769^T (DSM 44491 = type strains), SD880 (DSM 44500), SD910 (DSM 44501), SD925 (DSM 44502), SD914 and SD1000 which have been recently isolated from patient attending the Chest Unit at the Khartoum Teaching Hospital, Sudan[10]. Type strains of *N. farcinica* N267^T (ATCC 3318) and *N. asteroides* N317^T (ATCC 1924) in addition to clinical isolates: *N. farcinica* (SD72, SD1800, SD1819, SD1820, SD1828) and *N. asteroides* (SD1977, SD1978) from cattle and goats suffering from mastitis (Hamid et al., unpublished data).

The test strains were cultivated on Glucose Yeast Extract Agar medium (GYEA; glucose 10g, yeast extract 10g; agar 14 g; distilled water 1000 ml; pH 7.4) for 2-3 days at 37˚C and checked for purity prior to use. History and sources of *N. africana* strains were shown elsewhere[10].

In vitro antimicrobial Testing

The test strains were examined for their ability to grow on Mueller-Hinton agar medium in the presence of each of the 34 antimicrobial agents at various concentrations (Table 1). A homogeneous suspension giving an inoculum of 10^6-10^8 CFU/ml was used to streak the plates. The zone of inhibition was read after 36-48 h of incubation at 37 degrees C. Inhibition zone diameters were measured and recorded in millimeters. The organisms were then recorded as sensitive or resistant according to the zone size as shown by Bauer et al[11].

RESULTS

Percentages of susceptibility and resistance of *N. africana* to the 34 antibiotics and antimicrobial agents are shown in Table 2 and summarized in Figure 1. The results indicated that all *N. africana* isolates were sensitive to ciprofloxacin (5 μg/ml), clindamycin (10 μg/ml), fusidic acid

(10 µg/ml), gentamycin (10 µg/ml), imipenem (10 µg/ml), tobramycin (10 µg/ml), amikacin (20 µg/ml), doxycycline (30 µg/ml), minocycline (30 µg/ml), and vancomycine (30 µg/ml).

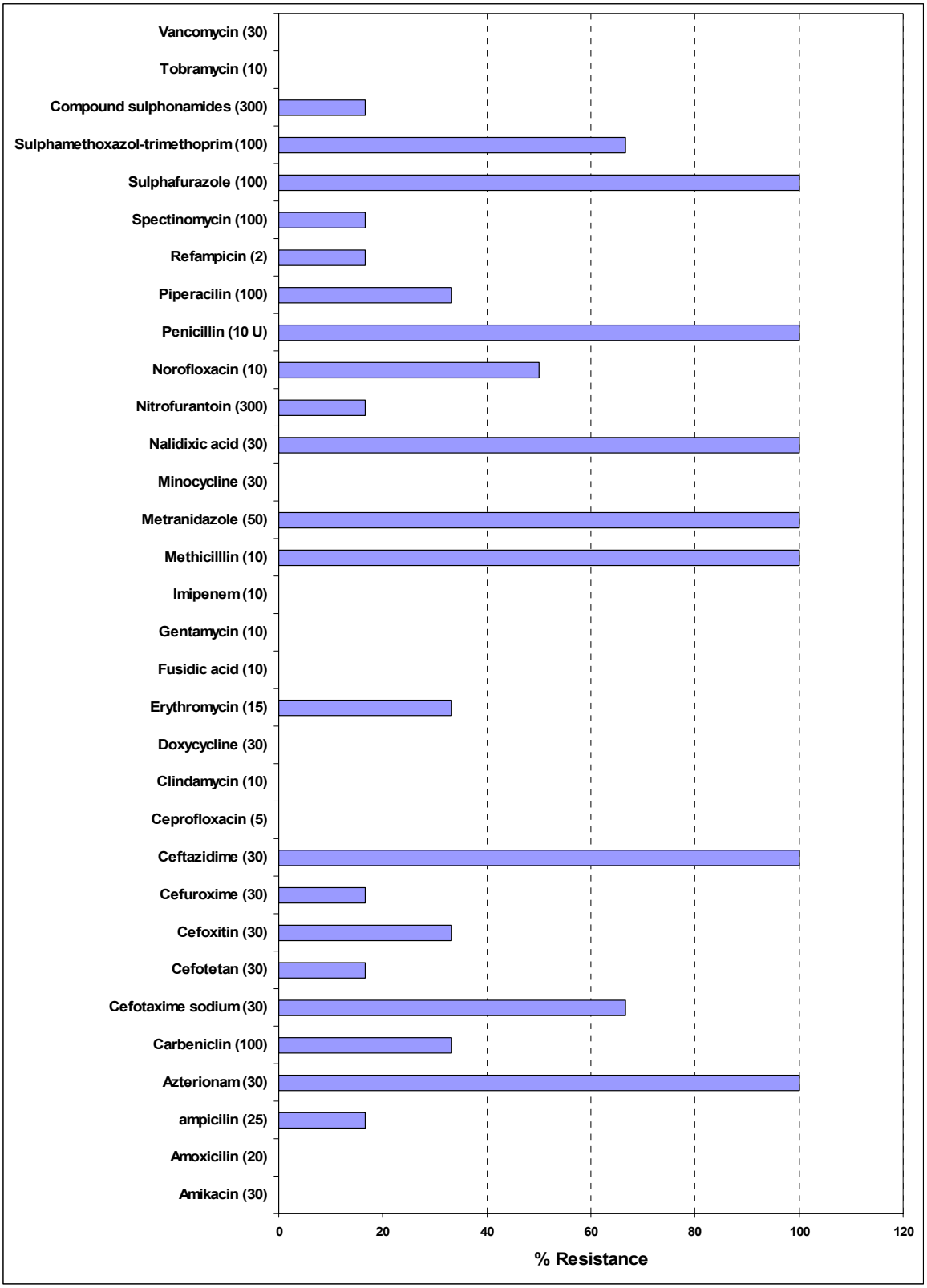

Figure 1. Resistant pattern of Nocardia africana to some antibiotics and antimicrobial agents

On the other hands *N. africana* isolates were found to be resistant to compound sulfonamides (300µg/ml), sulphafurazole (100µg/ml), metronidazole (50µg/ml), aztereonam (30µg/ml), cefotetan (30µg/ml) nalidixic acid (30µg/ml) and penicillin G (10 units).

Variable results to remaining antibiotics and antimicrobial agents were obtained as shown in Table 1 and Figure 1.

Table 1. Percentages of resistant strains of Nocadia africana and related species to some antimicrobial agents

Species Agent (µg/ ml)	*N. africana* (n= 6)	*N. asteroides* (n= 3)	*N.farcinica* (n=6)
Amikacin (30)	0.0	0.0	0.0
Amoxicilin (20)	0.0	0.0	0.0
Ampicilin (10)	16.6	0.0	33.3
ampicilin (25)	16.6	0.0	83.3
Azterionam (30)	100	100	100
Carbeniclin (100)	33.3	0.0	33.3
Cefotaxime sodium (30)	66.7	33.3	66.7
Cefotetan (30)	16.6	33.3	83.3
Cefoxitin (30)	33.3	0.0	33.3
Cefuroxime (30)	16.6	0.0	66.7
Ceftazidime (30)	100	33.3	100
Cprofloxacin (5)	0.0	0.0	0.0
Clindamycin (10)	0.0	0.0	0.0
Doxycycline (30)	0.0	0.0	0.0
Erythromycin (15)	33.3	0.0	0.0
Fusidic acid (10)	0.0	0.0	0.0
Gentamicin (10)	0.0	0.0	0.0
Imipenem (10)	0.0	0.0	0.0
Methicilllin (10)	100	100	100
Metranidazole (50)	100	100	100
Minocycline (30)	0.0	0.0	0.0
Nalidixic acid (30)	100	33.3	100
Nitrofurantoin (300)	16.6	0.0	0.0
Norofloxacin (10)	50	0.0	0.0
Penicillin (2 U)	100	66.7	100
Penicillin (10 U)	100	100	100
Piperacilin (100)	33.3	0.0	66.7
Refampicin (2)	16.6	0.0	33.3
Spectinomycin (100)	16.6	0.0	16.6

Sulphafurazole (100)	100	66.7	100
Sulphamethoxazol-trimethoprim (100)	66.7	33.3	66.7
Compound sulphonamides (300)	16.6	100	83.3
Tobramycin (10)	0.0	0.0	16.6
Vancomycin (30)	0.0	0.0	0.0

In comparison, all other tested *N. farcinica* and *N. asteroides* strains showed 100% susceptibility to ciprofloxacin (5μg/ml), amikacin (20μg/ml), amoxicillin (30μg/ml), clindamycin (10μg/ml), doxycycline (30μg/ml), fusidic acid (10 μg/ml), gentamycin (10 μg/ml), imipenem (10μg/ml) and tobramycin (10μg/ml). The two species, however, showed clear resistance to azteronam (30μg/ml), methicillin (10 μg/ml), metronidazole (50μg/ml), and penicillin (10 units). The majority *N. farcinica* strains were resistant to ampicillin (25μg/ml), cefotetan ((30μg/ml) and compound sulphonamides (300μg/ml).

DISCUSSION

Nocardiae are pathogens commonly found in soil worldwide, and they cause mostly opportunistic infections in humans and animals, complicating both immuno-suppressed states and primary diseases. Nocardiosis is difficult to diagnose because of its non-specific symptoms, which manifest as cutaneous and sub-cutaneous infections, lung symptoms and the dissemination through the bloodstream to other organs. A general feature of nocardia infections is their resistance to a wide range of the antibiotics tested[12,6-9].

In the present study notable resistance of *N. africana* was observed to sulphamethoxazole-trimethoprim (25μg/ml), cefotaxime sodium (30μg/ml), ampicillin (10 and 25μg/ml), ceftazidime (30μg/ml) and to a lesser extent to rifampicin (16.6%). These findings concur with most of the previous similar studies from other parts of the world and with different species of *Nocardia*. This resistance may be explained by the tough cell wall of nocardiae and the presence of large concentration of mycolic acids.

Although sulphamethoxazole-trimethoprim is commonly used for treatment of nocardiosis, our results showed significant resistance (70%) in line with other previous studies[13]. *N. africana* was found sensitive to amikacin, gentamycicn, doxycycline, minocycline, imipenem and vancomycin. These results concur with previous findings[12,6,14,7-9].

Torres et al recommended trimethoprim-sulfamethoxazole or amikacin combined with imipenem or amoxicillin-clavulanate for the treatment of *N. farcinica* infections[15]. This substantiates previous findings by Steingrube et al who demonstrated that *Nocardia brasiliensis* is susceptible to amoxicillin-clavulanic acid and that its beta-lactamases are inhibited *in vitro* by clavulanic acid[8]. In the present study *N. africana* was inhibited by amoxicillin without the incorporation of clavulanic acid (Table 1), but in agreement with other studies on pathogenic nocardiae, the resistance of *N. africana* to penicillin, methicillin and ampicillin was evident. Wallace et al found resistance to cefotaxime and cefamandole in 19% of 200 clinical *N. asteroides* isolates[9]. Similarly *N. africana* exhibit a clear resistance to cephalosporins such as cefotaxime sodium (30μg/ml) and ceftazidime (30μg/ml).

CONCLUSION

Among the best antibiotics found to act consistently against nocardiae both in the present study and previous ones are amikacin and minocycline. In addition *N. africana* revealed significant susceptibility to ciprofloxacin, clindamycin, fusidic acid, gentamycin, impenem, tobramycin, amikacin, amoxicillin, doxycycline, minocyclin and vancomycin, but resistance to sulphamethoxazole-trimethoprim, cefotaxime, penicillin, methicillin and ampicillin. Hence the drugs of choice can be selected form the susceptibility list, but we recommend ciprofloxacin, amikacin and doxycycline.

REFERENCES

1. Goodfellow M. *Nocardia* and related genera. In: Balows A, Duerden BI, eds. Topley and Wilson's Microbiology and Microbial Infection*s*. 9th edn. Vol. 2. London: Edward Arnold, 1998:463-89.
2. McNeil MM, Brown JM. The medically important aerobic actinomycetes: Epidemiology and microbiology. Clin Microbiol Rev 1994;7:357-417.
3. Corti ME, Villafane-Fioti MF. Nocardiosis: a review. Int J Infect Dis 2003;7: 243-50.
4. Laurent F, Mick V, Boiron P. Nocardia infections: clinical and biological aspects. Ann Biol Clin (Paris) 1999;57:545-54.
5. Wallace RJ Jr, Steele LC. Susceptibility testing of Nocardia species for the clinical laboratory. Diagn Microbiol Infect Dis 1988;9:155-66.
6. Gutmann L, Goldstein FW, Kitzis MD, et al. Susceptibility of Nocardia asteroides to 46 antibiotics, including 22 beta-lactams. Antimicrob Agents Chemother 1983;23:248-251.
7. Scopetti F, Iona E, Fattorini L, et al. Activity of antimicrobial drugs evaluated by agar dilution and radiometric methods against strains of *Nocardia asteroides* isolated in Italy from immunocompromised patients. J Chemother 1994;6:29-34.
8. Steingrube VA, Wallace RJ Jr, Brown BA, et al. Acquired resistance of Nocardia brasiliensis to clavulanic acid related to a change in beta-lactamase following therapy with amoxicillin-clavulanic acid. Antimicrob Agents Chemother 1991;35:524-8.
9. Wallace RJ Jr, Tsukamura M, Brown BA, et al. Cefotaxime-resistant Nocardia asteroides strains are isolates of the controversial species *Nocardia farcinica*. J Clin Microbiol 1990;28:2726-32.
10. Hamid ME, Maldonado L, Sharaf Eldin GS, et al. *Nocaria africana* sp. nov, a new pathogen isolated from patients with pulmonary infections. J Clin Microbiol 2002;39:625-30.
11. Bauer AW, Kirby WM, Sherris JC, et al. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966;45:493-6.
12. Boiron P, Provost F. *In-vitro* susceptibility testing of *Nocardia* spp and its taxonomic implication. J Antimic Chemoth 1988;22:623-9.
13. Khan Z, Al-Sayer H, Chugh TD, et al. Antimicrobial susceptibility profile of soil isolates of Nocardia asteroides from Kuwait. Clin Microbiol Infect 2000;6:94-8.
14. Hamid ME, Goodfellow M. In vitro antimicrobial susceptibility of bovine farcy organisms. Revue Elev Med Vet Pays Trop 1997;50:5-9.
15. Torres OH, Domingo P, Pericas R, et al. Infection caused by Nocardia farcinica: case report and review. Eur J Clin Microbiol Infect Dis 2000;19:205-12.