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Antibiotic Sensitivity of Some Nocardioform Bacteria and its Value as a Criterion for Taxonomy

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SUMMARY

We tested 151 strains representing the genera *Nocardia* and *Gordona*, the taxon '*Mycobacterium*' *rhodochrous* and a collection of strains isolated from bagasse for their *in vitro* susceptibility to 52 antimicrobial agents by using the impregnated filter-paper disc method. The 'bagasse' isolates were resistant to most of the antimicrobial agents but the nocardiae, gordonae and rhodochrous groups formed a series with increasing sensitivity. The nocardiae were inhibited by low concentrations of a number of antibiotics, notably erythromycin, miconazole, gentamycin and tobramycin, which may be of value in treating Nocardia infections. Certain antibiotics, at suitable concentrations, provided data of value for the classification and identification of nocardioform bacteria.

INTRODUCTION

Despite recent improvements in the classification of nocardioform bacteria (Cross & Goodfellow, 1973) we still require good characters for their classification and identification. Antibiotic sensitivity tests have provided characters for the classification and identification of actinomycetes though nocardiae have rarely featured in such studies. Filter-paper discs impregnated with antibiotics at known concentrations have been found to inhibit streptomycetes differentially (Okami, 1956; Okami, Hashimoto & Suzuki, 1960; Williams, 1967), while in earlier studies Waksman & Lechevalier (1953) demonstrated that most Streptomyces species were sensitive to antibacterial, but not antifungal, antibiotics. Williams (1967) showed that antibiotic sensitivity patterns were reproducible and correlated well with results obtained using other taxonomic criteria; he recommended that such data be included in the description of species and in numerical taxonomic studies. Characters from sensitivity tests have been included in the description of the genus *Micropolyspora* (Lechevalier, Solotorovsky & McDurmont, 1961), of *Microellobosporia* sp. (Cross, Lechevalier & Lechevalier, 1963), and have also been weighted for the identification of mycobacteria (Kubica *et al.* 1970; Silcox & David, 1971; Stanford & Gunthorpe, 1971; Boisvert, 1973). Tubercle bacilli can almost always be distinguished from opportunist mycobacteria by the results of sensitivity tests alone (Marks, 1973).

Some of the classical tests used in the taxonomy of nocardiae and streptomycetes have been found unreliable (Goodfellow, 1971; Sneath & Johnson, 1972) and additional tests are required to replace them. Better criteria are also needed for numerical taxonomic studies, because inappropriate tests can introduce levels of 'noise' that can seriously distort similarity coefficients (Sneath & Johnson, 1972). Friedman & MacLowry (1973) have shown that antibiotic sensitivity tests can provide data for the computer identification of clinically significant isolates.

In a numerical study carried out primarily on streptomycetes, Hill & Silvestri (1962) obtained 16 characters from antibiotic sensitivity tests by means of the impregnated-disc method. The results obtained with four of the antibiotics were considered to have a presumptive diagnostic value, and one of the sensitivity characters was used to construct a diagnostic key. Less success was found in a similar study on oral actinomycetes (Melville, 1965), for the test strains were inhibited by the five antibiotics used. In this work, the antibiotics, each at a concentration of 10 mg/100 ml, were poured into ditches cut in the solid medium which was then inoculated. A further approach was used by Krasilnikov & Agre (1964) when they cross-streaked known antibiotic-producing strains of streptomycetes with Thermopolyspora cultures and noted the inhibition of the latter. A few antibiotic sensitivity tests have occasionally been included in overall similarity studies on mycobacteria (Miková, Chládková & Kubín, 1973) and nocardiae (Bradley, 1971; Kurup & Schmitt, 1973). In the latter study the sensitivity tests were found to be particularly useful in differentiating the subgroups of *Nocardia asteroides* recovered.

A much larger number of antibiotics have been tested for their *in vitro* activity against nocardiae in studies where the emphasis was on chemotherapy. Most of these studies have provided little data of taxonomic value because the test strains have usually been restricted to a few poorly described clinical isolates (Wilson & Williams, 1966; Black & McNellis, 1970; Orfanakis, Wilcox & Smith, 1972). In similar studies Bach, Sabath & Finland (1973*b*) and Lerner & Baum (1973) included many well-described strains but, as these were primarily *Nocardia asteroides*, the taxonomic implications of their investigations were limited.

Although little attempt has been made to determine the antibiotic sensitivity patterns of nocardiae and related organisms it seems probable that such studies would provide useful taxonomic data. The data should also be of value to clinicians who are still faced with combating a sizeable mortality rate due to Nocardia infections. Accordingly, in the work reported here, well-classified strains of *Nocardia asteroides*, *N. brasiliensis*, *N. caviae* and '*Mycobacterium*' *rhodochrous* have been tested against examples of the common classes of antibiotics and a number of standard antimicrobial agents by using the impregnated filter-paper disc method. Representatives of the proposed taxon *Gordona* (Tsukamura, 1971), together with strains isolated from sugar cane bagasse provisionally classified as Nocardia (Lacey, 1974) were included for comparison.

METHODS

Organisms. In the initial studies 57 strains were chosen in an attempt to represent the range of variation found in the genera *Nocardia* and *Gordona*, and in the 'bagasse' and rhodochrous taxa (Table 1). All of the strains were maintained on yeast extract agar at room temperature.

A laboratory culture of *Staphylococcus aureus* was included for the specific purpose of ensuring that the antibiotic discs retained their activity after storage and during incubation.

Medium. Tests were carried out on Diagnostic Sensitivity Test (DST) agar (Oxoid, CM261). All of the cultures grew well on this medium, which had the additional advantage of being clear when solidified thereby ensuring that results could be read easily and accurately.

Preliminary examination of test procedure. The sensitivity of a number of test strains against a few of the antibiotics was examined by using the following methods: (i) Sterile antibiotics were incorporated into DST broth which was then inoculated with a 0·1 ml suspension of the test strain. By this method it was difficult to estimate the amount of

Table 1. *Strains used in the initial studies*

Strain		Source
N663	*Gordona aurantiaca*	NCTC 10741. Type strain
N654	*G. bronchialis*	NCTC 10667. ATCC 25592. Type strain: Sputum
N660	*G. bronchialis*	H. Mordarska, Hirszfeld's Institute, Wroclaw, Poland, T1. NCTC 10667. ATCC 25592
N661	*G. bronchialis*	H. Mordarska, T2. Sputum
N655	*Gordona rubra*	NCTC 10668. ATCC 25593. Soil
N657	*G. rubra*	H. Mordarska, T3. NCTC 10668. ATCC 25593
N658	*G. rubra*	H. Mordarska, T4. Soil
N656	*Gordona terrae*	NCTC 10669. ATCC 25594. Soil
N659	*G. terrae*	H. Mordarska, T5. NCTC 10669. ATCC 25594
N662	*G. terrae*	H. Mordarska, T6. Soil
N13	*Nocardia asteroides*	NCTC 8595. ATCC 14759. Cluster 1A.* Pus of empyema
N76	*N. asteroides*	S. T. Williams, University of Liverpool, E13; NCTC 6761. Cluster 1E*
N100	*N. asteroides*	R. E. Gordon, University of Rutgers, U.S.A., 652. Cluster 1A*
N102	*N. asteroides*	R. E. Gordon, 653. Type strain of *N. rhodnii*. Cluster 1A*
N216	*N. asteroides*	J. E. Thiemann, Lepetit S.p.a. Milan, Italy, S547. Cluster 1B*
N317	*N. asteroides*	ATCC 19247. Suggested type strain. Cluster 1D
N364	*N. asteroides*	M. Tsukamura, Chubu Chest Hospital. Aichi. Japan, R399. ATCC 23824
N485	*N. asteroides*	L. Ajello, Center for Disease Control, Atlanta, U.S.A., 45-1002-70. Cranial cavity
N486	*N. asteroides*	L. Ajello, 45-1005-70. Cervix
N565	*N. asteroides*	J. Lacey, Rothamsted Experimental Station, Harpenden, 1915. Sputum
N14	*Nocardia brasiliensis*	NCTC 10300. ATCC 19295. Cluster 5*
N48	*N. brasiliensis*	R. E. Gordon, 744. ATCC 19295. Cluster 5*
N213	*N. brasiliensis*	J. E. Thiemann, S581. Cluster 5*
N318	*N. brasiliensis*	ATCC 19296. Type strain. Cluster 5*
N465	*N. brasiliensis*	F. Mariat, Institut Pasteur, Paris, France, 701
N476	*N. brasiliensis*	A. Gonzáles Ochoa, Instituto de Salubridad y Enfermedades Tropicales, Mexico City, 4205. Mycetoma
N477	*N. brasiliensis*	A. Gonzáles Ochoa, 4204. Mycetoma
N517	*N. brasiliensis*	L. Ajello, 45-217-71
N557	*N. brasiliensis*	J. Lacey, 196. Mycetoma
N560	*N. brasiliensis*	J. Lacey, 199. Mycetoma
N561	*N. brasiliensis*	J. Lacey, 1910. Mycetoma
N3	*Nocardia caviae*	NCIB 9631. Cluster 2A*
N21	*N. caviae*	CCM 197. Cluster 2B*
N36	*N. caviae*	NCTC 1934. ATCC 14629. Type strain. Cluster 2B*. Middle ear of guinea pig
N231	*N. caviae*	R. J. Olds, Pathology Department, University of Cambridge, CN749. Cluster 2A. Isolated from a dachshund. Cluster 2B*
N232	*N. caviae*	R. J. Olds, CN751. Isolated from a corgi. Cluster 2B*
N313 to 314	*N. caviae*	F. Mariat, 751; 771. Cluster 2B
N369	*N. caviae*	M. Tsukamura, R1315
N5	*'Mycobacterium' rhodochrous*	NCIB 9701. Cluster 14C*
N7	*'M.' rhodochrous*	NCIB 10027. Cluster 14E*
N11	*'M.' rhodochrous*	NCIB 9158. Type strain of *N. erythropolis*. Cluster 14D*
N31	*'M.' rhodochrous*	R. E. Gordon, W21. Cluster 14B*
N53	*'M.' rhodochrous*	NCIB 8147; ATCC 11048. Type strain of *Jensenia canicruria*. Cluster 14D*
N54	*'M.' rhodochrous*	ATCC 13808. Cluster 14C*
N66	*'M.' rhodochrous*	NCTC 8139. Cluster 14E*
N84	*'M.' rhodochrous*	ATCC 4273. Cluster 14C*
N108	*'M.' rhodochrous*	R. E. Gordon, A4277; ATCC 4277. Cluster 14D*
N124	*'M.' rhodochrous*	CBS 330.61. Type strain of *N. opaca*. Cluster 14E*
N239	*'M.' rhodochrous*	NCTC 8727. Cluster 14C*
N240	*'M.' rhodochrous*	NCTC 8571. Cluster 14C*
N324	*'M.' rhodochrous*	ATCC 15998. Cluster 14A*

Table 1 (*cont.*)

Strain		Source	Strain		Source
N325	'M.' *rhodochrous*	G. Castelnuova, Instituto Superiori Di Sanita, Rome, 906B. Cluster 14A*	N389	*Nocardia* spp.	J. Lacey, A206; from mouldy bagasse
N326	'M.' *rhodochrous*	G. Castelnuova, 107. Cluster 14A*	N624	*Nocardia* spp.	J. Lacey, A1140
			N626	*Nocardia* spp.	J. Lacey, A1147

* Clusters recovered by Goodfellow (1971).

ATCC, American Type Culture Collection, Rockville, Maryland, U.S.A. CBS, Centraalbureau voor Schimmelcultures, Baarn, Netherlands. CCM, Czechoslovak Collection of Micro-organisms, J. E. Purkyne University, Brno, Czechoslovakia. NCIB, National Collection of Industrial Bacteria, Aberdeen. NCTC, National Collection of Type Cultures, London.

growth and impossible to decide upon the optimal time for reading the tests. (ii) Sterile antibiotics were incorporated into DST agar and the test organisms streaked onto the surface. After various periods of incubation the surface growth was compared with that on control plates without antibiotics. The uneven or patchy growth of organisms on both test and control plates caused considerable difficulties in reading the results. (iii) Antibiotics incorporated into filter-paper discs were placed onto DST agar seeded with a suspension of the test strain. In this method susceptible strains were easily recognized by the appearance of inhibition zones around discs. Because of its advantages over methods (i) and (ii) this technique was used in all subsequent work.

Preparation of inoculum. Organisms were inoculated into DST broth and incubated at 37 °C for 7 days, when most strains showed good surface growth. Fine bacterial suspensions were obtained by thorough mixing on a Vortex mixer.

Preparation of plates. DST broth suspensions (0·1 ml) were pipetted onto dried plates of 4 mm deep DST agar and spread with a sterile bent glass rod. Dried antibiotic discs were placed aseptically onto the agar, six per plate.

Selection of incubation period. Test plates were read daily for 7 days, then after 14 and 21 days at 37 °C. For most strains clear stable inhibition zones were formed after 7 days. Occasionally, mainly with rhodochrous strains, weak inhibition zones became completely obscured by overgrowth after 5 to 7 days of incubation. Since the definitive results were read after 7 days of incubation such strains were considered to be resistant.

Preparation of filter-paper discs. Discs of filter paper (Whatman No. 1), 5 mm in diam, were sterilized in Universal bottles and then soaked for 2 min in sterile antibiotic solutions at concentrations of 1, 10, 50, 100, 500 and 1000 μg/ml. The excess solution was drained off and the discs rapidly freeze-dried, under vacuum, over P_2O_5 until dry. The dried discs were stored at 4 °C until needed.

The antibiotics and antimicrobial agents were sterilized by filtration through Seitz pads in Hemmings filters (T. B. Ford Ltd, Loudwater, Buckinghamshire). Most of these compounds were provided as powders which were readily dissolved in sterile water, though added drops of acetone, ethanol, methanol or sodium hydroxide were necessary to bring a few antibiotics into solution.

Antibiotics and other antimicrobial agents. By means of the procedure described the test strains were screened against the 52 antimicrobial agents listed as follows. (i) Aminoglycosides: gentamycin sulphate (Roussel); kanamycin sulphate (Bristol Laboratories Ltd); neomycin sulphate (Sigma); streptomycin sulphate (Dista Products Ltd); tobramycin (Lilly). (ii) Anasamacrolides: rifampicin (Lepetit). (iii) Cephalosporins: cephaloridine

(Glaxo); sodium cephalothin (Lilly). (iv) Chloramphenicol: chloramphenicol (Carlo Erba). (v) Cycloserine: cycloserine (Lilly). (vi) Lincomycins: clindamycin hydrochloride (Upjohn); lincomycin hydrochloride (Upjohn). (vii) Macrolides: erythromycin (Lilly); oleandomycin (Pfizer). (viii) Novobiocin: sodium novobiocin (Glaxo). (ix) Penicillins: amoxicillin (Bencard); sodium ampicillin (Beecham Laboratories); sodium benzyl-penicillin (Dista Products Ltd); sodium epicillin (Squibb); sodium methicillin (Beecham Laboratories). (x) Peptides: calcium amphomycin (Bristol Laboratories); bacitracin (Sigma); capreomycin sulphate (Dista Products Ltd); colomycin (Pharmax); polymixin B sulphate (Burroughs Wellcome). (xi) Polyenes: mycostatin (Squibb); pimaricin (Brocades). (xii) Steroids: fusidic acid (Leo Laboratories). (xiii) Tetracyclines: chlortetracycline hydrochloride (Lederle); demethylchlortetracycline hydrochloride (Lederle); doxycycline (Pfizer); methacycline hydrochloride (Pfizer); minocycline hydrochloride (Lederle); oxytetracycline hydrochloride (Pfizer); tetracycline hydrochloride (Lederle). (xiv) Vancomycin: vancomycin hydrochloride (Lilly). (xv) Anti-tubercular drugs: ethambutol hydrochloride (Lederle); ethionamide (May and Baker); para-aminosalicylic acid (Sigma); thioacetozone (Boots). (xvi) Nitrofurans: Furazolidone (Smith, Kline and French); nitrofurantoin (Smith, Kline and French); nitrofurazone (Smith, Kline and French). (xvii) Sulphonamides: sulphadiazine (May and Baker); sulphafurazole (May and Baker); sulphamerazine (May and Baker); sulphamethoxypyridazine (Lederle); sulphathiazole (May and Baker). (xviii) Additional compounds: Dapsone (4,4 diaminodiphenylsulphone, Cox and Co. Ltd); miconazole nitrate (Janssen Pharmaceutica); nalidixic acid (Bayer Products); septrin (Burroughs Wellcome).

Antimicrobial agents of possible taxonomic value. An additional 93 strains (Table 2) were screened against concentrations of those antimicrobial agents considered to have a presumptive taxonomic significance (see Table 5, below).

Reproducibility studies. After approximately one year a proportion of the tests were repeated; 100 of the test organisms were screened against selected concentrations of six antibiotics (Table 3).

RESULTS

Reproducibility studies

It can be seen from Table 3 that the sensitivity patterns were reproducible.

Preliminary studies

The susceptibilities of the test strains to the 52 antimicrobial agents are recorded in Tables 4 and 5. It is evident that most of the compounds showed some antagonistic effect, though the antifungal antibiotics, mycostatin and pimaricin, did not inhibit any of the strains and with a few exceptions this was also true for the anti-tubercular drugs and the nitrofurans. In contrast miconazole, which has both antifungal and antibacterial properties, inhibited all of the strains at low concentrations.

The antibacterial antibiotics were most active against the rhodochrous strains, and to a progressively lesser extent against the gordonae and nocardiae, but had comparatively little effect upon the 'bagasse' isolates. The latter were, however, susceptible to high concentrations of the cephalosporins, some of the tetracyclines, and to benzyl-penicillin, dapsone, fusidic acid, septrin and the sulphonamides.

The nocardiae, gordonae and rhodochrous strains were most susceptible to the aminoglycoside antibiotics, gentamycin and tobramycin being the most effective. Erythromycin and the tetracycline analogues, particularly doxycycline and minocycline, also showed

Table 2. *Additional strains tested using the selected antibiotics*

Strain		Source
N96	*Nocardia asteroides*	R. E. Gordon, W3300. Cluster 1A*
N97	*N. asteroides*	R. E. Gordon, N659. Cluster 1A*
N98	*N. asteroides*	R. E. Gordon, 618. Cluster 1A*
N105	*N. asteroides*	R. E. Gordon, 9969. ATCC 9969. Cluster 1A*. Generalized actinomycosis
N228	*N. asteroides*	NCMB 1470. Cluster 1B*. Piscine tuberculosis
N233	*N. asteroides*	R. Olds, CN750. Cluster 1B*. Cow's milk
N457	*N. asteroides*	L. Ajello, 45-783-70. Isolated from fish-tank water
N483	*N. asteroides*	L. Ajello, 45-995-70. Isolated from a cow
N487	*N. asteroides*	L. Ajello, 45-1002-70. Sputum
N489	*N. asteroides*	L. Ajello, 45-1007-70. Gastric washing
N490	*N. asteroides*	L. Ajello, 45-1091-70. Isolated from a dog
N491	*N. asteroides*	L. Ajello, 45-1066-70. Brain tissue
N493	*N. asteroides*	L. Ajello, 45-1126-70. Sputum
N509	*N. asteroides*	L. Ajello, 45-1109-70
N511	*N. asteroides*	L. Ajello, 45-32-71
N512	*N. asteroides*	L. Ajello, 45-54-71
N518	*N. asteroides*	L. Ajello, 45-231-71
N519	*N. asteroides*	L. Ajello, 45-246-71
N520	*N. asteroides*	L. Ajello, 45-247-71
N566	*N. asteroides*	J. Lacey, 1916. Pus
N619	*N. asteroides*	J. Lacey, A265
N118	*Nocardia brasiliensis*	CBS 438.64. Cluster 5.* Multiple sinus
N212	*N. brasiliensis*	J. E. Thiemann, S580. Cluster 5*
N367	*N. brasiliensis*	M. Tsukamura, R432
N368	*N. brasiliensis*	M. Tsukamura, R887
N428	*N. brasiliensis*	R. E. Gordon, 1336
N429	*N. brasiliensis*	R. E. Gordon, 731
N438	*N. brasiliensis*	R. E. Gordon, 1108. Arm pustule
N439	*N. brasiliensis*	R. E. Gordon, 3488
N467	*N. brasiliensis*	F. Mariat, 708
N468	*N. brasiliensis*	F. Mariat, 723
N469	*N. brasiliensis*	F. Mariat, 748
N470	*N. brasiliensis*	A. Gonzáles-Ochoa, 4060. Mycetoma, foot
N471	*N. brasiliensis*	A. Gonzáles-Ochoa, 4115. Mycetoma, lower leg
N473	*N. brasiliensis*	A. Gonzáles-Ochoa, 4216. Mycetoma, chest
N475	*N. brasiliensis*	A. Gonzáles-Ochoa, 4023. Mycetoma, forearm
N480	*N. brasiliensis*	J. A. Serrano, Universidad de los Andes, Merida, Venezuela, 1548
N481	*N. brasiliensis*	J. A. Serrano, M168
N482	*N. brasiliensis*	L. Ajello, 45-944-70. Scapular abscess
N488	*N. brasiliensis*	L. Ajello, 45-1012-70
N530	*N. brasiliensis*	L. Ajello, 45-247-71
N130	*Nocardia caviae*	CBS 226.38. Cluster 2A*
N430	*N. caviae*	R. E. Gordon, 1370. Isolated from a dog's lung
N431	*N. caviae*	R. E. Gordon, 737. Bone marrow
N432	*N. caviae*	R. E. Gordon, 416
N442	*N. caviae*	R. E. Gordon, 424
N562	*N. caviae*	J. E. Lacey, 1911
N563	*N. caviae*	J. E. Lacey, 1912
N22	'*Mycobacterium*' *rhodochrous*	CCM 3245. ATCC 4273. Cluster 14C*
N26	'*M.*' *rhodochrous*	CCM 198. Cluster 14C*
N27	'*M.*' *rhodochrous*	CCM 278. Cluster 14C*
N28	'*M.*' *rhodochrous*	CCM 269. Cluster 14C*
N55	'*M.*' *rhodochrous*	R. E. Gordon, 817. Cluster 14C*
N56	'*M.*' *rhodochrous*	R. E. Gordon, 1256. Cluster 14D*
N61	'*M.*' *rhodochrous*	R. E. Gordon, W3408. ATCC 4273. Cluster 14C*
N63	'*M.*' *rhodochrous*	R. E. Gordon, 463. Cluster 14D*
N65	'*M.*' *rhodochrous*	R. E. Gordon, A7698. Cluster 14D*
N327, N328	'*M.*' *rhodochrous*	G. Castelnuova, 906; 350. Cluster 14A*
N361, N362	'*M.*' *rhodochrous*	M. Tsukamura, M1; M75. ATCC 9356. (*N. rubra*)
N420 to N423	'*M.*' *rhodochrous*	A. Tacquet, University of Lille, France, 906; 906B; 107; 330. (*N. pellegrino*)
N444 to N445	'*M.*' *rhodochrous*	Hill, University of Edinburgh, A/O; B/O. (*N. rhodnii*)
N448, N449	'*M.*' *rhodochrous*	R. Bönicke, Forschungsinstitut Borstel, West Germany, SN5104, SN5105 (*N. pellegrino*)
N450	'*M.*' *rhodochrous*	R. Bönicke, SN5303 (*N. corallina*)
N453	'*M.*' *rhodochrous*	R. Bönicke, SN5206 (*N. rubra*)
N385, N386	*Nocardia* spp.	J. Lacey, A153; A197. Isolated from mouldy bagasse

Table 2 (*cont.*)

Strain		Source	Strain		Source
N570	*Nocardia* spp.	J. Lacey, A185	N622, N623	*Nocardia* spp.	J. Lacey, A994 to 5
N572 to N584	*Nocardia* spp.	J. Lacey, A1132 to A1139; A1141, A1142; A1144 to A1146	N625	*Nocardia* spp.	J. Lacey, A1143
N617, N618	*Nocardia* spp.	J. Lacey, A195; A216	N640	*Nocardia* spp.	J. Lacey, A205

* Clusters recovered by Goodfellow (1971).
NCMB, National Collection of Marine Bacteria, Aberdeen; for other abbreviations see Table 1.

Table 3. *Reproducibility studies with selected antibiotics in filter-paper discs*

Antibiotic	Concentration of antibiotic solution (μg/ml)	Replication (%)
Kanamycin	10	93
Neomycin	50	90
Streptomycin	100	96
Erythromycin	50	93
Novobiocin	50	92
Vancomycin	50	93

considerable activity against these organisms. None of the remaining antimicrobial agents were as effective against the nocardiae as the aminoglycosides and tetracyclines. The cephalosporins, penicillins and peptide antibiotics inhibited most of the rhodochrous strains, but apart from capreomycin and polymixin B, they had little effect on the nocardiae. The sulphonamides, however, were more active against the nocardiae than against gordonae or rhodochrous strains. The gordonae were far less susceptible to the penicillins, cephalothin, oleandomycin and rifampicin than the rhodochrous strains.

Microbial agents of possible taxonomic value

The susceptibilities of all the test strains to the selected concentrations of 21 antimicrobial agents, chosen after the initial studies, are shown in Table 6. It is evident that few of the characters behaved with absolute consistency for a taxon but this is hardly surprising, especially for the genus *Gordona*, the rhodochrous taxon and *N. asteroides*, all of which undoubtedly cover a comparatively wide range of variation. Sufficient representatives of the three homogeneous subgroups of the rhodochrous taxon (Goodfellow, 1971) were included to make it possible to calculate results on a subgroup basis. No qualitative difference was found in the susceptibility patterns of the subgroups or *Gordona* spp. There was, therefore, sufficient consistency within and between taxa for several characters to be extracted from Table 6 and weighted for identification. Characters which were 80 % or more positive within a taxon were designated +, those 20 % sensitive or below −, and those with values between 21 and 79 % were scored d for doubtful (Table 7). It is apparent that several of the antibiotic sensitivity characters should be of value in differentiating Nocardia *sensu stricto* from the other nocardioform taxa studied.

Table 4. *Percentage of nocardiae strains inhibited by antimicrobial agents in filter-paper discs*

Species ...	*N. asteroides*						*N. brasiliensis*						*N. caviae*					
No. strains ...	10						11						8					
Concentration of antimicrobial solution (μg/ml) ...	1000	500	100	50	10	1	1000	500	100	50	10	1	1000	500	100	50	10	1
Gentamycin	90	90	90	80	70	30	100	100	100	100	91	27	100	100	100	100	100	13
Kanamycin	40	40	10	10	0	0	18	18	9	9	9	0	100	100	100	100	100	25
Neomycin	100	100	70	70	20	0	82	55	27	9	9	0	63	38	25	25	25	0
Streptomycin	50	30	10	10	0	0	73	55	18	0	0	0	50	13	0	0	0	0
Tobramycin	90	90	80	80	80	20	100	100	100	100	82	18	100	88	50	50	25	13
Rifampicin	40	20	10	0	0	0	27	9	0	0	0	0	50	38	25	13	0	0
Cephalothin	20	10	10	0	0	0	18	0	0	0	0	0	13	13	13	13	0	0
Chloramphenicol	20	10	0	0	0	0	27	9	9	9	0	0	25	0	0	0	0	0
Clindamycin	30	20	10	0	0	0	100	38	0	0	0	0	88	63	25	0	0	0
Lincomycin	20	20	10	0	0	0	36	18	9	9	0	0	100	50	0	0	0	0
Erythromycin	100	100	80	40	20	10	100	100	100	73	18	0	100	100	63	25	13	0
Novobiocin	20	0	0	0	0	0	36	27	18	9	9	0	88	88	88	75	13	0
Ampicillin	60	30	10	0	0	0	9	0	0	0	0	0	38	25	13	13	0	0
Benzyl penicillin	40	20	10	0	0	0	27	9	9	0	0	0	25	13	13	0	0	0
Capreomycin	100	90	70	20	0	0	9	9	9	0	0	0	75	50	0	0	0	0
Polymixin B	70	40	30	10	0	0	55	46	9	0	0	0	38	25	25	0	0	0
Fusidic acid	100	100	50	10	0	0	46	18	0	0	0	0	25	13	0	0	0	0
Chlortetracycline	90	90	10	0	0	0	82	73	18	9	0	0	100	100	38	25	13	0
Demethylchlor-tetracycline	90	80	40	10	0	0	82	73	9	0	0	0	100	100	38	25	0	0
Doxycycline	90	70	50	20	0	0	64	46	18	0	0	0	100	88	75	63	0	0
Methacycline	90	70	10	0	0	0	64	46	18	0	0	0	75	50	25	13	0	0
Minocycline	100	70	60	50	0	0	100	91	82	9	9	0	100	100	100	75	38	0
Vancomycin	80	50	40	30	0	0	27	18	9	0	0	0	25	25	0	0	0	0
Dapsone	50	20	10	0	0	0	55	27	0	0	0	0	25	13	0	0	0	0
Sulphadiazine	30	10	0	0	0	0	46	36	0	0	0	0	75	13	0	0	0	0
Sulphafurazole	70	40	10	0	0	0	91	91	27	9	0	0	100	63	13	0	0	0
Sulphamerazine	60	20	0	0	0	0	46	36	0	0	0	0	50	38	0	0	0	0
Sulphamethoxypyri-dazine	90	50	0	0	0	0	82	73	36	0	0	0	75	38	13	0	0	0
Sulphathiazole	90	90	50	40	0	0	91	82	36	18	0	0	100	75	25	13	0	0
Nalidixic acid	0	0	0	0	0	0	91	0	0	0	0	0	0	0	0	0	0	0
Septrin	90	60	30	10	10	0	82	64	36	9	9	0	100	50	13	13	0	0
Miconazole	100	100	100	100	40	0	100	100	100	91	9	0	100	100	100	100	13	0

No inhibition with mycostatin, pimaricin, PAS, thiacetozone, nitrofurazone or nitrofurantoin. A few strains were inhibited by the higher concentrations of cephaloridine, cycloserine, oleandomycin, amoxicillin, epicillin, methicillin, amphomycin, bacitracin, colomycin, oxytetracycline, tetracycline, ethambutol, ethionamide and furazolidone.

DISCUSSION

Reliable characters are essential for a sound classification which separates taxa having a high phenetic similarity. Although improvements have been made recently in the classification of Nocardia and Mycobacterium some of the characters used in the description of these taxa have been found unreliable (Goodfellow, 1973). There is therefore a need for additional criteria to be used in the taxonomy of nocardioform organisms. New tests should be reproducible, easy to do, should give clear-cut results and must not depend upon the use of chemicals or equipment beyond the means of diagnostic laboratories. Antibiotic sensitivity tests meet all of these requirements. Cowan (1970) considered that the description of a bacterium was rarely complete without reference to its antibiotic sensitivity pattern.

Table 5. *Percentage of Gordona, 'Mycobacterium' rhodochrous and 'bagasse' strains inhibited by antimicrobial agents in filter-paper discs*

Taxon ...	*Gordona*						*'M.' Rhodochrous*						*'Bagasse'*					
No. strains ...	10						10						3					
Concentration of antimicrobial solution (μg/ml) ...	1000	500	100	50	10	1	1000	500	100	50	10	1	1000	500	100	50	10	1
Gentamycin	100	100	100	90	60	0	100	100	100	100	100	34	0	0	0	0	0	0
Kanamycin	100	90	90	90	30	0	100	87	80	80	40	0	0	0	0	0	0	0
Neomycin	100	100	100	100	90	0	100	100	94	94	94	7	0	0	0	0	0	0
Streptomycin	80	80	60	60	0	0	100	100	94	80	20	0	66	33	0	0	0	0
Tobramycin	100	100	90	90	80	0	100	100	100	100	80	20	0	0	0	0	0	0
Rifampicin	90	70	30	10	0	0	100	100	94	94	74	54	66	33	0	0	0	0
Cephalordine	100	100	90	20	0	0	100	100	80	67	20	0	100	100	66	66	0	0
Cephalothin	20	0	0	0	0	0	94	80	47	13	0	0	100	66	0	0	0	0
Chloramphenicol	70	40	10	0	0	0	94	47	13	0	0	0	0	0	0	0	0	0
Clindamycin	60	60	0	0	0	0	100	80	34	13	0	0	33	0	0	0	0	0
Lincomycin	80	70	60	40	0	0	100	100	94	67	7	0	0	0	0	0	0	0
Erythromycin	100	100	90	90	40	0	100	100	94	80	67	20	66	33	0	0	0	0
Oleandomycin	50	40	10	0	0	0	94	87	80	27	0	0	66	0	0	0	0	0
Novobiocin	0	0	0	0	0	0	47	47	47	40	7	0	0	0	0	0	0	0
Amoxicillin	50	30	0	0	0	0	87	74	40	27	0	0	66	0	0	0	0	0
Ampicillin	0	0	0	0	0	0	87	80	60	20	7	0	0	0	0	0	0	0
Benzyl penicillin	70	0	0	0	0	0	100	94	27	7	0	0	100	0	0	0	0	0
Epicillin	0	0	0	0	0	0	87	60	27	0	0	0	0	0	0	0	0	0
Bacitracin	70	20	0	0	0	0	67	60	34	13	7	0	0	0	0	0	0	0
Capreomycin	100	100	20	10	0	0	94	87	47	27	0	0	0	0	0	0	0	0
Polymixin B	80	80	0	0	0	0	94	94	27	13	0	0	33	33	0	0	0	0
Fusidic acid	100	100	80	60	20	0	100	100	87	80	34	7	100	100	66	0	0	0
Chlortetracycline	90	80	0	0	0	0	100	100	74	60	0	0	100	66	33	0	0	0
Demethylchlor-tetracycline	90	80	10	0	0	0	100	100	87	54	20	0	100	100	33	33	0	0
Doxycycline	100	100	80	40	0	0	100	94	94	34	0	0	33	33	0	0	0	0
Methacycline	80	70	10	0	0	0	100	100	40	0	0	0	100	33	0	0	0	0
Minocycline	100	100	80	30	30	0	100	100	100	87	27	0	66	66	66	33	0	0
Oxytetracycline	60	50	0	0	0	0	67	40	7	0	0	0	0	0	0	0	0	0
Vancomycin	90	90	70	40	0	0	100	100	100	100	34	0	33	33	0	0	0	0
Dapsone	60	50	0	0	0	0	47	34	0	0	0	0	100	100	66	0	0	0
Ethambutol	0	0	0	0	0	0	74	47	13	0	0	0	0	0	0	0	0	0
Sulphafurzaole	60	40	20	0	0	0	47	27	7	7	0	0	66	0	0	0	0	0
Sulphamerazine	30	0	0	0	0	0	20	13	0	0	0	0	100	0	0	0	0	0
Sulphamethoxypyri-diazine	70	20	10	0	0	0	34	34	7	7	7	0	100	0	0	0	0	0
Sulphathiazole	90	90	40	10	0	0	67	40	7	7	7	0	100	100	0	0	0	0
Septrin	30	0	0	0	0	0	27	13	7	0	0	0	100	100	0	0	0	0
Miconazole	100	100	100	100	20	0	100	100	100	87	0	0	100	100	100	100	0	0

No inhibition with mycostatin, pimaricin, ethionamide, PAS, thiacetozone, nitrofurantoin or nalidixic acid. A few strains inhibited by the higher concentrations of cycloserine, methicillin, amphomycin, colomycin tetracycline, furazolidone, nitrofurazone and sulphadiazine.

The potential value of antibiotic sensitivity tests in the taxonomy of nocardiae and allied organisms can be assessed from the data presented. By using the impregnated filter-paper disc method, results of diagnostic value were obtained without reference being made to the shape or size of inhibition zones. When diagnosis is based on the positive or negative response of the test organism, and not on differences in the size of inhibition zones, good replication is found. In this study better reproducibility could perhaps have been obtained by standardizing inocula, but even without this refinement the results of the reproducibility

Table 6. *Percentage of test strains inhibited by antimicrobial agents in filter-paper discs*

Taxon ...		*N. asteroides*	*N. brasiliensis*	*N. caviae*	*Gordona*	'*M.*' *rhodochrous*	'Bagasse'
No. strains ...		30	28	15	11	38	25
Antimicrobial agents	Concentration (μg/ml)						
Gentamycin	10	43	94	66	55	91	0
Gentamycin	100	80	100	100	100	97	0
Kanamycin	10	3	0	80	36	36	0
Streptomycin	100	17	11	0	55	94	0
Neomycin	50	76	11	20	100	94	0
Tobramycin	50	66	86	53	91	94	0
Tobramycin	100	69	86	59	91	94	0
Rifampicin	50	0	0	7	9	91	8
Cephaloridine	100	7	0	0	91	83	40
Lincomycin	100	3	18	0	63	75	0
Erythromycin	50	43	54	20	91	83	0
Oleandomycin	100	0	7	0	9	60	0
Novobiocin	50	7	7	80	0	34	0
Amoxicillin	500	10	0	7	27	60	24
Capreomycin	100	59	11	0	18	60	4
Polymixin B	100	7	7	13	0	13	0
Fusidic acid	100	40	0	0	73	91	84
Demethychlor-tetracycline	500	59	36	93	82	94	96
Doxycycline	100	36	22	73	73	55	64
Minocycline	50	33	25	73	27	70	80
Vancomycin	50	13	4	0	36	100	44
Dapsone	500	23	36	20	45	26	92
Septrin	500	43	76	33	0	36	100

Table 7. *Characters weighted for the identification of Nocardia and allied taxa*

		Taxon					
Antibiotic	Concentration (μg/ml)	*N. asteroides*	*N. brasiliensis*	*N. caviae*	*Gordona*	'*M.*' *rhodochrous*	'Bagasse'
Gentamycin	100	+	+	+	+	+	−
Kanamycin	10	−	−	+	d	d	−
Neomycin	50	d	−	−	+	+	−
Streptomycin	100	−	−	−	d	+	−
Tobramycin	50	d	+	d	+	+	−
Rifampicin	50	−	−	−	−	+	−
Cephaloridine	100	−	−	−	+	+	d
Novobiocin	50	−	−	+	−	d	−
Fusidic acid	100	d	−	−	d	+	+
Vancomycin	50	−	−	−	d	+	d

+, > 80 % positive; −, > 80 % negative; d, values between 21 and 79 %.

studies fell well within the guidelines recommended for numerical taxonomic studies (Sneath & Johnson, 1972).

Consistent and marked differences in *in vitro* antimicrobial sensitivity patterns were observed between strains representing the taxa studied. The 'bagasse' isolates were conspicuous by their resistance to nearly all the antimicrobial agents and this suggests that they are incorrectly classified in the genus *Nocardia*. In contrast, the rhodochrous strains, and to a lesser extent the gordonae, were much more sensitive to the antimicrobial agents than the nocardiae. This result adds weight to the view that the rhodochrous taxon is distinct from, and equivalent in rank to, the genus *Nocardia*.

Several workers (Bach *et al.* 1973*b*; Lerner & Baum, 1973; Wilson & Williams, 1966) have found wide intra-specific variation patterns in antibiotic sensitivity studies carried out primarily on strains of *Nocardia asteroides*. The results of the present study are much more encouraging and this may be attributable to improvements in the taxonomy of the genus *Nocardia* which influence the selection of test strains. However, Bach *et al.* (1973*b*) also reported pronounced variations in the response of well-selected strains of *N. asteroides* to almost all of 45 antimicrobial agents. It is, however, acknowledged that this taxon is heterogeneous (Tsukamura, 1969; Goodfellow, 1971); indeed Kurup & Schmitt (1973) found differences in antibiotic sensitivity between the four subgroups of *N. asteroides* they recovered. In the work reported here strains of *N. caviae* were found to be susceptible to low concentrations of kanamycin but *N. asteroides* and *N. brasiliensis* were resistant. The results also confirmed the observation of Lerner & Baum (1973) who found that *N. brasiliensis* was more susceptible to erythromycin and gentamycin, and more resistant to capreomycin, than *N. asteroides*. Antibiotic sensitivity tests do, therefore, seem to be of value in the subgeneric classification of the genus.

Nocardia asteroides is an opportunistic pathogen, particularly in patients whose resistance has been undermined by immunosuppressive drugs, corticosteroids, anti-metabolites or neoplastic disease (Young, Armstrong, Blevins & Lieberman, 1971). Mortality in treated patients ranges from 10 % in uncomplicated cases of nocardiosis to nearly 90 % in cases of cerebral nocardiosis (Hoeprich, Brandt & Parker, 1968). There are also problems in treating the more serious disease, actinomycete mycetoma, caused primarily by *N. brasiliensis*. Sulphonamide thereapy is still widely used to treat Nocardia infections but it is not always successful, and cannot be applied when patients are hypersensitive to the drugs. It is for these reasons that other antimicrobial agents are now used, in conjunction with, or as an alternative to, the sulphonamides. It is by no means clear which additional or alternative drugs should be recommended; cycloserine (Rhoades, Riley & Muchmore, 1961; Hoeprich *et al.* 1968), ampicillin (Orfanakis *et al.* 1972), septrin (Baikie, MacDonald & Mundy, 1970), and erythromycin and sulphone (Vasarinsh, 1968) have been tried. It can be concluded from the present study that erythromycin, miconazole nitrate and the aminoglycoside antibiotics, especially gentamycin and tobramycin, might be effective in treating Nocardia infections. In addition minocycline, the other chemically related tetracycline analogues, clindamycin and septrin showed *in vitro* activities comparable to that of the sulphonamides. The results with minocycline were of particular interest for it was shown recently (Bach, Gold & Findland, 1973*a*) that this antibiotic was as effective as sulphadiazine in eliminating a large inoculum of *Nocardia asteroides* from mice and prolonging their survival after intraperitoneal injection of a suspension in mucin.

The difficulties associated in correlating *in vitro* results with the presumptive therapeutic value of antimicrobial agents are legion. There are several reports of discrepancies found between the *in vitro* sensitivities of nocardiae to antibiotics and the effectiveness of these agents in treating experimental nocardiosis (Runyon, 1951; Sanford, Hatten & Fordtran, 1957–1958). Such studies are hampered because details of the virulence and pathogenicity of nocardiae are poorly understood, and because of the difficulties in simulating in laboratory animals the disease as it appears in man (Mason & Hathaway, 1969; Wilson & Williams, 1966). *In vitro* data must therefore be treated with care, but it is nevertheless clear that the laboratory evaluation of antimicrobial agents is an advance over therapeutic management on a trial and error basis. Improved therapy for Nocardia infections will depend upon the results of well co-ordinated clinical and bacteriological studies.

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