BACTERIAL CHARACTERISATION

Identification and antibiotic susceptibility of *Nocardia farcinica* and *N. nova* in the UK

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Nocardia asteroides has long been recognised as a heterogeneous group of organisms. The description and identification of two new subgroups, *N. farcinica* and *N. nova*, in other countries encouraged us to re-examine a collection of *N. asteroides* isolates from the UK. Of 73 clinical isolates identified as *N. asteroides* from different parts of England and Wales during 1991–1993, and now subjected to further differentiation tests by the Mycobacterial/Nocardial Reference Laboratory, 15 (20.5%) were identified as *N. farcinica* based on three out of four characteristics: growth property, acetamide production, rhamnose assimilation and a distinct antibiogram. No isolates were identified as *N. nova*. A revised identification and susceptibility system has significant clinical and taxonomic implications. Its introduction will improve speciation, identify antibiotic resistance and influence the choice of safer alternative therapy for patients infected with *Nocardia* spp. in the UK.

Introduction

Members of the genus *Nocardia* form a complex group of organisms, of which *N. asteroides*, *N. brasiliensis* and *N. otitidiscaviarum* are responsible for most cases of human nocardiosis. DNA homology and antimicrobial susceptibility studies have shown that *N. asteroides* is a heterogeneous group of organisms [1]. In the latest edition of *Bergey's Manual of Systematic Bacteriology* [2], two new subgroups of *N. asteroides* were recognised, of which *N. farcinica* was described as a valid species and *N. nova* was listed as species *incertae sedis*.

The species characteristics of the genus *Nocardia* are differentiated in *Bergey's Manual* on the basis of phenotypic tests comprising tests for the hydrolysis of casein, tyrosine, xanthine, hypoxanthine and testosterone, the degradation of urease and aesculin, acid production from carbohydrates, decarboxylation of citrate, production of nitrate reductase and growth characteristics [3]. These tests differentiate the species *N. asteroides*, *N. brasiliensis*, *N. otitidiscaviarum*, *N. amarae*, *N. brevicatena*, *N. carnea*, *N. vacinii* and *N. transvalensis*. Among the 'species' *N. asteroides*, acid production from rhamnose in Goodfellow's selection

of tests helps differentiate *N. farcinica* from *N. asteroides*. However, a single test is insufficient for identification of a species. In 1990, Wallace *et al.* described additional growth and biochemical characteristics for the identification of *N. farcinica* [4]. They are: comparable growth on tryptic soya agar slopes at 45°C and 35°C, acid production from rhamnose, and the ability to utilise acetamide as a nitrogen and carbon source. A year later Wallace and other co-authors also described the clinical and laboratory features of *N. nova* [5].

In an earlier study, Wallace *et al.* [6] had shown that the heterogeneous group *N. asteroides* displayed five distinct antimicrobial susceptibility patterns that may be useful taxonomically; 'drug pattern type 5' showed resistance to cephalosporins and tobramycin and conformed to *N. farcinica*, while 'drug pattern type 3' showed susceptibility to ampicillin and erythromycin and was consistent with *N. nova*. While these patterns are useful for species differentiation, they also influence the choice of antibiotic therapy for nocardiosis in patients with drug allergy or a poor clinical response.

The Mycobacterium/Nocardia Reference Laboratory at Dulwich Public Health Laboratory/King's College School of Medicine and Dentistry receives strains of *Nocardia* and related genera from England and Wales for identification and susceptibility testing. Because of

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the emerging taxonomic and clinical significance of the newly recognised subgroups a collection of *N. asteroides* clinical isolates from 1991 to 1993 were re-examined to ascertain the identification and susceptibility patterns of '*N. asteroides*', *N. farcinica* and *N. nova* in the UK.

Materials and methods

Organisms

During 1991–1993, 102 clinical isolates were identified as *N. asteroides* and stored on Lowenstein-Jensen slopes. Isolates were recovered from slopes by making a suspension of the colonies in 1 ml of Middlebrook 7H9 broth with Middlebrook OADC enrichment medium (Becton Dickinson, USA). A total of 84 isolates was recovered, of which 11 were contaminated, and therefore excluded, leaving 73 clinical isolates for this study. In addition, reference strains *N. asteroides*

NCTC 11293 and *N. farcinica* NCTC 11134 were examined to aid species identification.

Tests

Bergey's Manual classifies the genus *Nocardia* by a compilation of phenotypic tests [2]. Table 1 lists the set of tests that was previously used in the Reference Laboratory to differentiate the genus *Nocardia*. Four additional tests shown in Table 2 for the identification of *N. farcinica*, i.e., comparable growth on Tryptone Soya Agar (TSA; Unipath) after incubation for 3 days at 35°C and 45°C, utilisation of acetamide as a nitrogen and carbon source, production of acid from rhamnose, and a single test of arylsulphatase for the identification of *N. nova*, were used to identify the two new subgroups from the heterogeneous group of organisms, namely '*N. asteroides*'. These were prepared in-house by methods described previously [7]. Acid production was determined on Columbia agar base with rhamnose

Table 1. Standard tests to differentiate *Nocardia* species [2]

Tests	<i>N. asteroides</i>	<i>N. braziliensis</i>	<i>N. otitidis-caviarum</i>	<i>N. amarae</i>	<i>N. brevicatena</i>	<i>N. carnae</i>	<i>N. vaccinii</i>	<i>N. transvalensis</i>
Hydrolysis of								
casein	–	+	–	–	–	–	–	–
tyrosine	–	+	d	–	–	–	–	+
xanthine	–	–	+	–	–	–	–	+
hypoxanthine	–	+	+	–	–	–	–	+
aesculin	+	+	+	+	+	+	+	+
urea	+	+	+	+	–	–	+	+
nitrate	+	+	+	+	–	+	+	+
Growth in presence of lysozyme	+	+	+	–	+	+	+	+

+, ≥ 90% strains positive; –, ≥ 90% strains negative; d, ≥ 11–89% strains positive.

Table 2. Additional biochemical, growth and susceptibility tests used for differentiating *N. asteroides*, *N. farcinica* and *N. nova*

Tests	<i>N. asteroides</i>	<i>N. farcinica</i>	<i>N. nova</i>
Hydrolysis of testosterone	–	–	–
Presence of			
arylsulphatase	–	–	+
β-galactosidase	–	–	–
β-lactamase	–	–	–
Growth on TSA			
at 35°C	+	+	+
at 45°C	+	+	+
Utilisation of			
acetamide as N and C source	–	+	–
citrate as sole C source	–	–	–
Production of acid from rhamnose	–	+	–
Antibiotic susceptibility disk content (μg) and (sensitive zone diameter in mm)			
sulphamethaxazole 25 (> 21)	R	S	S
cefotaxime 30 (> 20)	R	S	S
netilmicin 10 (> 21)	S	S	S
ceftazidime 30 (> 20)	R	R	S
sulphonamide compound 300 (> 21)	S	S	S
imipenem 10 (> 20)	S	S	S
ciprofloxacin 5 (> 26)	S	S	R
ampicillin 25 (> 16)	S	R	S
erythromycin 15 (> 20)	S	S	S
tobramycin 10 (> 20)	S	R	d
ceftriaxone 30 (> 20)	S	S	S
amikacin 10 (> 21)	S	S	S

+, ≥ 90% strains positive; –, ≥ 90% strains negative; d, ≥ 11–89% strains positive; R, resistant; S, sensitive; TSA, tryptone soya agar; N, nitrogen; C, carbon.

and phenol red as indicator and was considered positive when the upper portion of the agar slope turned dark pink. The test for production of acid was controlled by parallel incubation of a slope without rhamnose for all the isolates tested. The utilisation of acetamide as a nitrogen and carbon source was shown by a yellow colouration of the entire slope with bromothymol blue indicator when the agar slope contained acetamide, but showed no colour reaction with a negative slope. *N. nova* was identified by the presence of the enzyme arylsulphatase, which produces a deep red colour when phenolphthalein is released from its disulphate on addition of an alkali.

Antibiotic susceptibility tests

The taxonomic differentiation of Mycobacteriaceae and Nocardaceae and of their individual species requires additional antibiotic susceptibility testing. Disk diffusion susceptibility tests were performed on all 73 *Nocardia* isolates on IsoSensitest Agar (Unipath) with lysed horse blood 5%. Table 2 shows the 12 commercially prepared antibiotic disks (Unipath) that were selected on the basis of previous in-vitro studies and their use in clinical practice, disk content and zone diameters used for interpretation. Disks containing β -lactam antibiotics and imipenem were not placed contiguously to avoid errors of enzyme induction [8]. 'Drug pattern type 5' indicates resistance to tobramycin and cephalosporins but susceptibility to amikacin, ciprofloxacin and imipenem, and this was used to identify *N. fariginica*. Strains susceptible to ampicillin and erythromycin are referred to as 'drug pattern type 3' and were identified as *N. nova*. *Staphylococcus aureus* NCTC 6571 was used as the control organism for susceptibility testing.

Cluster analysis

The results obtained from biochemical, growth and susceptibility characteristics (30 variables) on all 73 clinical isolates and three reference strains were subjected to cluster analysis by the unweighted-average pair group method (Multivariate Statistical Programme: W. L. Kovach, Aberystwyth, Wales).

Results

Fig. 1 shows the dendrogram generated from the cluster analysis of the results of the 30 tests (variables) shown in Tables 1 and 2 for all the clinical and reference strains. It indicates that the strains cluster around four distinct groups – I, II, III and IV – and have an overall percentage similarity between 80 and 100%. The reference strain *N. fariginica* was in group I, and groups III and IV contained the reference strains of *N. asteroides* and *N. nova*, respectively. Group II contained a single clinical strain. The most striking feature of the dendrogram is that, although all groups are

closely related, group I showed many inter-strain differences whereas group III contained two major subgroups and showed fewer variations between strains. The inter-group differences were also minimal with a 72.9% similarity.

Table 3 compares the distinguishing characteristics and patterns observed for the reference strain of *N. fariginica* and the 15 clinical isolates in group I. Fourteen different patterns were observed, of which only one was identical to that of the reference strain. All strains utilised acetamide, the majority (93%) showed comparable growth on TSA at 35°C and 45°C after incubation for 3 days, 80% produced acid from rhamnose and 67% hydrolysed nitrates. All strains were resistant to ceftazidime, up to 93% resistant to cefotaxime and almost one third were resistant to ceftriaxone, whilst 73% were resistant to tobramycin. Unlike the reference strain, only one third of clinical isolates produced β -lactamase.

Table 4 compares the differentiating characteristics and patterns observed for the reference strain *N. asteroides* and the 57 clinical isolates in group III. Seventeen different patterns were observed among the clinical and reference strains. None of the clinical isolates showed a pattern identical to that of the reference strain. The only common characteristic in the majority (96%) was hydrolysis of urea. Unlike the reference strain, nearly half (44%) the isolates hydrolysed nitrates, 53% grew on TSA at 45°C and, unexpectedly, 89% of isolates utilised acetamide. The majority of the isolates were susceptible to the antibiotics chosen; only 15% were resistant to β -lactam agents and also to sulphonamides.

Discussion

The taxonomic classification of *Nocardia* spp. has changed over the last 10 years. Cluster analysis of results of biochemical, growth and susceptibility tests confirmed the heterogeneity of the species *N. asteroides* and the presence of the subgroup *N. fariginica* in the collection of isolates of the Reference Laboratory. Despite inter-strain variations observed among the *N. fariginica* isolates, three additional tests were found to be useful in the identification of this species: 93% of strains showed comparable growth on TSA at 35°C and 45°C after incubation for 3 days, 100% utilised acetamide as the sole carbon and nitrogen source and 80% produced acid from rhamnose, indicating the reliability of these tests for identification. 'Drug pattern 5' did not reliably characterise *N. fariginica*. The arylsulphatase test and 'drug pattern 3' were not helpful in the identification of *N. nova*.

Antibiotic resistance in *Nocardia* spp. generally reflects intrinsic resistance, as most infections are environmentally acquired, but extrinsic resistance has

Fig. 1. Dendrogram generated by the unweighted-average pair group method of the biochemical, growth and susceptibility characteristics from Tables 1 and 2 for 73 clinical isolates and two reference strains (RS). The pattern numbers in small roman numerals and number of isolates with similar patterns (in brackets) correspond to those shown in Tables 3 and 4. The clinical isolates and reference strains cluster around four distinct groups (I-IV)

Table 3. Selection of distinguishing characteristics and patterns observed for reference strain and clinical isolates in group I

Tests	Reference strain <i>N. fariginica</i>	Pattern of results with clinical isolates in group I														Percentage of isolates (results)
		i	ii	iii	iv	v	vi	vii	viii	ix	x	xi	xii	xiii	xiv	
Hydrolysis of aesculin	—	—	—	—	—	—	—	—	+	—	—	—	—	—	+	13 (+)
Reduction of nitrate	+	+	+	+	+	+	+	+	+	—	+	—	—	—	—	67 (+)
Presence of β -galactosidase	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	7 (+)
β -lactamase	—	+	+	—	—	—	—	—	—	+	—	—	—	—	+	33 (+)
Growth on TSA at 45°C	+	+	+	+	+	+	+	+	+	—	+	+	+	+	+	93 (+)
Utilisation of acetamide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100 (+)
Production of acid from rhamnose	+	+	+	+	—	+	+	+	+	—	—	+	+	+	+	80 (+)
Susceptibility to ceftazidime	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	100 (R)
tobramycin	R	R	R	R	R	R	R	R	R	R	S	S	S	S	R	73 (R)
ceftriaxone	S	R	S	S	R	R	S	S	S	S	S	S	S	S	R	27 (R)
cefotaxime	S	R	R	R	R	R	R	S	R	R	R	R	R	R	R	93 (R)
sulphonamide	S	R	R	R	R	R	S	S	S	R	R	R	R	R	R	47 (R)
sulphamethoxazole	S	R	R	R	R	R	S	S	S	R	R	R	R	R	R	80 (R)
Number of clinical isolates with this pattern	...	2	1	1	1	1	1	1	1	1	1	1	1	1	1	

Table 4. Selection of distinguishing characteristics and patterns observed for reference strain and clinical isolates in group III

Tests	Reference strain <i>N. asteroides</i>	Pattern of results with clinical isolates in group III																Percentage of isolates (results)
		i	ii	iii	iv	v	vi	vii	viii	ix	x	xi	xii	xiii	xiv	xv	xvi	
Presence of urease	+	—	+	+	+	+	+	+	+	—	—	+	+	+	+	+	+	96 (+)
arylsulphatase	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	4 (+)
Reduction of nitrate	—	+	+	+	+	+	—	—	—	—	+	+	—	+	+	+	+	44 (+)
Growth on TSA at 45°C	—	—	—	+	+	+	—	+	—	—	+	+	—	+	+	+	+	53 (+)
Utilisation of acetamide	—	—	—	+	+	+	+	+	—	+	—	+	+	—	+	+	+	89 (+)
Production of acid from rhamnose	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	+	4 (+)
Susceptibility to ciprofloxacin	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	2 (R)
amoxicillin	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	7 (R)
ceftazidime	R	S	S	S	S	S	S	S	S	S	R	R	S	R	R	R	S	11 (R)
erythromycin	S	S	S	S	S	S	S	S	S	S	S	R	R	S	R	S	S	7 (R)
imipenem	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	2 (R)
sulphonamide	R	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	2 (R)
sulphamethoxazole	R	S	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	4 (R)
ceftriaxone	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	5 (R)
cefotaxime	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	5 (R)
Number of clinical isolates with this pattern	...	1	1	1	15	1	21	5	2	1	1	3	1	1	1	1	1	

also been shown in > 90% of clinical isolates of *N. asteroides* by the presence of β -lactamases. The β -lactamases in *Nocardia* spp. vary in number, content, enzyme activities and enzyme induction [8]. A single β -lactamase pattern that correlated with resistance to broad-spectrum cephalosporins was identified by isoelectric focusing in 'drug pattern 5' only [6]. There is evidence of variable cephalosporin resistance in these clinical isolates of *N. fariginica* and *N. asteroides* which may be explained by the presence of many different β -lactamases in a given clinical isolate. The possibility that differences in β -lactamases may relate to varying β -lactam resistance needs to be investigated further.

Antibiotic susceptibility testing is unreliable as a taxonomic tool for *N. fariginica*. Greater inter-strain

variability was observed when susceptibility patterns were included. Although all strains of *N. fariginica* in group I were resistant to ceftazidime, and 93% were resistant to cefotaxime, only 27% were resistant to ceftriaxone. Cephalosporin resistance (5–11%) was also observed in *N. asteroides* 'species', suggesting that it cannot be used as a marker for the identification of *N. fariginica*. A prompt and early confirmation of cephalosporin resistance is of clinical importance, as these agents are used increasingly as alternatives to sulphonamides for the treatment of nocardiosis, particularly when problems of drug allergy, a cerebral abscess or poor clinical response are encountered [9].

Epidemiological data on the incidence of infection with both species is beginning to emerge. *N. fariginica* was found to be the prevailing pathogenic actinomycete in

Germany during 1979–1991 [10] and accounted for one quarter of all *Nocardia* isolates in France during 1989–1990 [11]. Reports of sporadic cases and larger studies that relate *N. farcinica* and *N. nova* with clinical disease have been published from the USA where, in one study, 19% of isolates identified initially as *N. asteroides* were subsequently shown to be *N. farcinica* [4, 5]. However, there have been no case reports or prevalence studies of *N. farcinica* and *N. nova* in the UK. *N. farcinica* has been reported as a pathogen in lung infection and disseminated infection predominantly in immunocompromised patients [4, 12]. Reports of infection with *N. farcinica* in AIDS patients are also increasing [13], but its precise incidence will only emerge when laboratory identification of this species becomes more widespread. Characterisation of a large UK collection of clinical isolates from 1991 to 1993 in the present study led to the conclusion that 15 (20.5%) of the 73 clinical isolates referred to the Reference Laboratory and previously identified as *N. asteroides* were in fact *N. farcinica*.

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