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Determination of antimicrobial susceptibility patterns of *Nocardia* spp. from clinical specimens by Etest

Y. Glupczynski[1], *C. Berhin*[1], *M. Janssens*[2] *and G. Wauters*[2]

[1]Department of Clinical Microbiology, UCL Mont-Godinne University Hospital, Yvoir, and [2]University of Louvain, Faculty of Medicine, Microbiology Unit, Brussels, Belgium

ABSTRACT

Susceptibilities to 11 antimicrobial agents were determined by Etest for 93 *Nocardia* isolates from clinical specimens and 15 type strains belonging to different *Nocardia* spp. All isolates were susceptible to trimethoprim-sulphamethoxazole, amikacin and linezolid, but susceptibilities of the various *Nocardia* spp. to β-lactams, aminoglycosides, ciprofloxacin and clarithromycin varied markedly. Overall, there was a good correlation between the drug resistance patterns and the species identification established by conventional phenotypic tests and 16S rDNA sequencing. Among the different species encountered, *Nocardia farcinica* and *Nocardia brasiliensis* displayed the most multiresistant profiles, with resistance to imipenem occurring mainly among isolates of *N. brasiliensis* and *Nocardia abscessus*. The species variability in susceptibility profiles and the numerous recent taxonomic changes means that in-vitro susceptibility tests may be a complementary tool for the identification of *Nocardia* isolates from human clinical specimens. Further studies on a larger number of species from more diverse geographical sources, including species that are found less commonly among clinical isolates, are required to validate and extend the results.

Keywords Antimicrobial susceptibility tests, diagnosis, Etests, identification, *Nocardia* spp., species identification

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INTRODUCTION

Nocardia spp. are isolated with increasing frequency from clinical specimens, and especially from immunocompromised patients [1]. The taxonomy of the genus *Nocardia* has been revised extensively during recent years, and a number of unnamed taxons have been delineated [2,3]. Within the genus, *Nocardia asteroides* has usually been considered to be the most frequent species isolated from clinical specimens [4–9]. However, this species has been shown to be heterogeneous (termed the *N. asteroides* complex) and has now been divided into several different species [2,3,10], and new species isolated from humans have also been described [11–13]. Identification of *Nocardia* isolates to the species level is difficult by means of routine phenotypic tests, but a simple identification scheme, based on a panel of nine conventional phenotypic and enzymatic tests, has been developed and validated for the rapid identification of the most common *Nocardia* spp. found in human clinical specimens [14]. Molecular tests using PCR and sequencing of the 16S rDNA gene have been advocated for the accurate identification of *Nocardia* isolates to the species level [2,10], and in-vitro susceptibility patterns have been shown to differ among *Nocardia* spp., and to allow the separation of the *N. asteroides* complex into six distinct groups [15].

Few in-vitro studies of the use of antimicrobial susceptibility profiles to identify the various *Nocardia* spp. have been carried out since the latest taxonomic changes [2,3]. The aim of the present study was to assess the drug susceptibility patterns of *Nocardia* spp. found in a large number of clinical

Corresponding author and reprint requests: Y. Glupczynski, Department of Clinical Microbiology, UCL Mont-Godinne University Hospital, B-5530 Yvoir, Belgium
E-mail: youri.glupczynski@skynet.be

specimens against a panel of antimicrobial agents by means of the Etest.

MATERIALS AND METHODS

Microorganisms

Ninety-three *Nocardia* isolates from human clinical specimens in Belgium were investigated, of which 83 were isolated between 1990 and 2005, and ten were isolated before 1990. The isolates were collected by 21 laboratories in various parts of Belgium. All the *Nocardia* isolates were included in the study in order to avoid any bias in selection. Only one isolate per patient was considered. Identification to the species level was by conventional biochemical tests (resistance to lysozyme, production of urease, hydrolysis of gelatin, degradation of casein, tyrosine, xanthine and hypoxanthine), acid production from rhamnose, citrate alkalinisation, enzymatic activities and growth at 45°C. In addition, the entire 16S rDNA gene of each isolate was sequenced and aligned with library sequences of type strains in the GenBank database [14].

Reference type strains of 15 different *Nocardia* spp. isolated from human clinical specimens were also tested for their susceptibilities: *N. asteroides* (NCTC 11293^T), *N. abscessus* (DSM 44432^T), *N. africana* (DSM 44491^T), *N. asiatica* (DSM 44668^T), *N. brasiliensis* (NCTC 11294^T), *N. carnea* (DSM 43397^T), *N. cyriacigeorgica* (DSM 44484^T), *N. farcinica* (DSM 43665^T), *N. niigatensis* (DSM 44670^T), *N. nova* (DSM 43207^T), *N. paucivorans* (DSM 44386^T), *N. pseudobrasiliensis* (DSM 44290^T), *N. otitidiscaviarum* (NCT1934^T), *N. transvalensis* (DSM 43405^T) and *N. veterana* (DSM 44445^T). *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212 and *Escherichia coli* ATCC 35218 (for amoxycillin-clavulanate only) were used as quality controls to monitor the antimicrobial susceptibility tests.

Inoculum preparation

The *Nocardia* isolates were subcultured twice on sheep blood agar to ensure purity. A large amount of growth was scraped from the second sheep blood agar plate, inoculated into 20 mL Mueller-Hinton (MH) broth, and incubated at 35°C for 24–48 h on a rotary shaker. Sterile glass beads (3 mm in diameter) were added to each flask to minimise the formation of clumps of nocardial growth during cultivation. In order to prevent the formation of irregular clumps, tubes were vortexed periodically at high speed for 2 min to achieve a uniform suspension, and vortexing was repeated before determination of ODs. The final inoculum was adjusted to an OD equivalent to a 1.0 MacFarland standard with a densitometer (Densimat; bioMérieux, Marcy-l'Etoile, France).

Antimicrobial susceptibility testing

MICs were determined by Etest (AB Biodisk, Solna, Sweden) on 150-mm MH agar plates inoculated by confluent swabbing using a Retro C80 automatic inoculating device (AB Biodisk). A maximum of five Etest strips were applied to each MH agar plate with the Simplex C76 Etest instrument (AB Biodisk). Plates were incubated at 35°C in ambient air and interpreted in accordance with the guidelines provided by the manufacturer. Results were recorded after 48 h (or after 72 h if growth was insufficient after 48 h).

Antimicrobial agents

The following antimicrobial agents were tested (concentration ranges in mg/L): ampicillin (0.016–256), amoxycillin + clavulanic acid 2 : 1 (0.016–256), piperacillin+tazobactam (0.016–256 + 4), ceftriaxone (0.016–256), imipenem (0.002–32), ciprofloxacin (0.002–32), tobramycin (0.016–256), amikacin (0.016–256), clarithromycin (0.016–256), linezolid (0.016–256), trimethoprim + sulphamethoxazole 1 : 19 (0.002–32) and minocycline (0.016–256).

β-Lactamase testing

β-Lactamase determination was by the nitrocefin disk method (Cefinase; Becton Dickinson, Erembodegem, Belgium). Colour change readings were taken for up to 1 h and were recorded as positive or negative. Isolates with negative test results were subcultured on MH agar plates on which amoxycillin + clavulanate disks were placed. After adequate growth, colonies growing closest to the zone of inhibition were retested for β-lactamase activity by the nitrocefin disk method as described above (to detect amoxycillin + clavulanate-induced β-lactamase).

Interpretation of susceptibility results

Results were interpreted as susceptible, intermediately-resistant or resistant according to the breakpoints recommended by the CLSI (NCCLS) for *Nocardia* and other aerobic actinomycetes [16], except for amoxycillin and piperacillin + tazobactam, for which CLSI interpretative criteria for Enterobacteriaceae were used to establish tentative breakpoint values [17].

RESULTS

The species distribution (*n*) of the clinical isolates was as follows: *N. farcinica* (41), *N. nova* (20), *N. cyriacigeorgica* (13), *N. brasiliensis* (6), *N. abscessus* (6), *N. paucivorans* (2), *N. asiatica* (1), *N. carnea* (1), *N. elegans* (1), *N. niigatensis* (1) and *N. veterana* (1). Thirty-nine isolates were from the respiratory tract, 18 from superficial abscesses or wounds, ten from blood, six from brain abscesses and one from cerebrospinal fluid. Nineteen isolates were of unknown origin.

All except six isolates (two *N. abscessus*, two *N. nova*, one *N. cyriacigeorgica*, one *N. paucivorans*) produced adequate growth on unsupplemented MHA and yielded ODs of ≥1.0 McFarland in ≤48 h at 35°C. Coarse clumping growth was common, but the addition of glass beads and periodic vortexing helped to break-up clumps and pellicles. For the six isolates mentioned above, the incubation period was extended to 72 h because of insufficient growth.

Growth inhibition ellipses were uniform and well-delineated, and the points of intersection with

the Etest strips were easy to determine. The MIC results for the 86 isolates belonging to the most common species are summarised in Table 1. All 93 *Nocardia* isolates from clinical specimens were susceptible to amikacin, trimethoprim + sulphamethoxazole and linezolid. Trimethoprim + sulphamethoxazole displayed the highest intrinsic activity on the basis of the MIC_{50} value

Table 1. Antimicrobial susceptibilities of 86[a] clinical isolates of various *Nocardia* spp. to 11 antimicrobial agents

	MIC range mg/L	MIC_{50} mg/L	MIC_{90} mg/L	% susceptible[b]
Nocardia farcinica (n = 41)				
Amoxycillin	0.38–128	16	48	9.7
Amoxycillin + clavulanate	0.5–4	2	3	100
Piperacillin + tazobactam	24- > 256	> 256	> 256	0
Ceftriaxone	0.38- > 256	96	> 256	22.0
Imipenem	0.047–3	0.75	1.5	100
Ciprofloxacin	0.047- > 32	0.75	8	53.7
Clarithromycin	1.5- > 256	16	32	4.9
Minocycline	0.047–4	2	4	12.1
Linezolid	0.047–6	1.5	3	100
TMP + SMX	0.016–2	0.064	0.75	100
Amikacin	0.38–2	0.75	1	100
Tobramycin	2–32	16	24	7.3
Nocardia nova (n = 20)				
Amoxycillin	0.032–1.5	0.125	0.5	100
Amoxycillin + clavulanate	1- > 256	8	> 256	50
Piperacillin + tazobactam	0.023- > 256	> 256	> 256	30
Ceftriaxone	0.032- > 256	2	32	70
Imipenem	0.002–1	0.032	0.064	100
Ciprofloxacin	8- > 32	> 32	> 32	0
Clarithromycin	< 0.016–0.064	0.016	0.032	100
Minocycline	0.25–6	1.5	3	35
Linezolid	0.25–3	1	2	100
TMP + SMX	0.064–0.75	0.25	0.75	100
Amikacin	0.016–0.25	0.064	0.25	100
Tobramycin	1–16	4	8	60
Nocardia cyriacigeorgica (n = 13)				
Amoxycillin	2- > 256	8	> 256	61.5
Amoxycillin + clavulanate	4–128	6	12	76.9
Piperacillin + tazobactam	> 256	> 256	> 256	0
Ceftriaxone	0.5–4	1	3	100
Imipenem	0.094–1.5	0.38	1.5	100
Ciprofloxacin	> 32	> 32	> 32	0
Clarithromycin	4- > 256	24	> 256	0
Minocycline	0.5–4	1.5	3	30.7
Linezolid	0.25–2	1	1.5	100
TMP + SMX	0.016–0.25	0.064	0.19	100
Amikacin	0.19–1	0.38	0.75	100
Tobramycin	< 0.016–0.19	0.032	0.064	100
Nocardia abscessus (n = 6)				
Amoxycillin	0.016–3	0.38	3	100
Amoxycillin + clavulanate	0.016–0.38	0.064	0.38	100
Piperacillin + tazobactam	1- > 256	8	> 256	50
Ceftriaxone	0.125–1.5	0.5	1.5	100
Imipenem	0.094- > 32	2	> 32	50
Ciprofloxacin	1.5- > 32	32	> 32	0
Clarithromycin	1.5–8	2	8	66.7
Minocycline	0.016–1	0.032	1	100
Linezolid	0.125–1.5	0.5	1.5	100
TMP + SMX	0.004–0.094	0.032	0.125	100
Amikacin	0.064–0.19	0.125	0.19	100
Tobramycin	0.016–0.5	0.094	0.5	100
Nocardia brasiliensis (n = 6)				
Amoxycillin	0.5–48	12	48	33.3
Amoxycillin + clavulanate	0.25–4	0.75	4	100
Piperacillin + tazobactam	6- > 256	> 256	> 256	16.6
Ceftriaxone	1- > 256	6	> 256	50
Imipenem	8- > 32	> 32	> 32	0
Ciprofloxacin	1.5- > 32	8	> 32	0
Clarithromycin	4- > 256	12	> 256	0
Minocycline	0.38–2	0.75	2	83.3
Linezolid	0.25–1.5	0.25	1.5	100
TMP + SMX	0.004–0.047	0.032	0.047	100
Amikacin	0.094–1	0.5	1	100
Tobramycin	< 0.016–0.064	0.032	0.064	100

[a]Susceptibility results for seven clinical isolates (*N. paucivorans*, 2; *N. carnea*, 1; *N. asiatica*, 1; *N. elegans*, 1; *N. niigatensis*, 1; *N. veterana*, 1) are not included in the table (see Results in the text).
[b]According to CLSI guidelines [16,17].
TMP + SMX, trimethoprim + sulphamethoxazole.

(0.064 mg/L), followed by amikacin (0.38 mg/L) and linezolid (1 mg/L). No resistance to minocycline was observed, but 70% of isolates had MICs in the intermediate susceptibility category (MIC_{50}, 2 mg/L). The activity of β-lactams was variable, with 89% of isolates being susceptible to imipenem, 84% to amoxycillin + clavulanate, 55% to ceftriaxone, 50% to amoxycillin (MIC ≤ 8 mg/L), and 9% to piperacillin + tazobactam (MIC ≤ 16 mg/L). Other antimicrobial agents with varying and species-dependant activity were tobramycin (52% susceptible), clarithromycin (32% susceptible) and ciprofloxacin (31% susceptible).

β-Lactam agents

All the *Nocardia* isolates, including the 15 reference strains, showed β-lactamase activity by the nitrocefin disk test. Among the clinical isolates, 73 showed a positive reaction within 5–10 min, and 14 showed a gradually positive reaction over a period of 1 h. For six isolates of *N. nova*, the nitrocefin test was initially negative, but became positive after re-testing colonies subcultured on MHA with amoxycillin + clavulanate.

Despite displaying β-lactamase activity, all *N. nova* isolates were susceptible to amoxycillin, while *c*.50% of the isolates belonging to this species were either intermediately-resistant or resistant to amoxicillin + clavulanate (Tables 1 and 2). The inhibitory activity of amoxycillin was 4->100-fold superior to that of amoxycillin + clavulanate for each individual isolate of *N. nova*. Conversely, over 90% of all *N. farcinica* isolates were resistant to amoxycillin, and all were uniformly susceptible to amoxycillin + clavulanate. Likewise, amoxycillin + clavulanate appeared to be significantly more active *in vitro* than amoxycillin alone against *N. brasiliensis* isolates (MIC_{50} 0.75 and 16 mg/L, respectively). Against *N. cyriacigeorgica* and *N. abscessus*, amoxycillin and amoxycillin + clavulanate had equal activity, although *N. cyriacigeorgica* was usually less susceptible (MIC_{50} 8 mg/L for both amoxycillin and amoxycillin + clavulanate) than *N. abscessus* (MIC_{50} 0.38 mg/L for amoxycillin and 0.064 mg/L for amoxycillin + clavulanate). Piperacillin + tazobactam did not display significant activity against most of the *Nocardia* spp. tested. Resistance to ceftriaxone was observed primarily in *N. farcinica* and, to a lesser extent, in *N. brasiliensis* and *N. nova* (Table 2). Resistance to imipenem was observed with all *N. brasiliensis* isolates, three of six *N. abscessus* isolates, and the single clinical isolate of *N. niigatensis*.

Among the less common species, two isolates of *Nocardia paucivorans* and one isolate of *N. carnea* were susceptible to all β-lactams tested, except piperacillin + tazobactam. One isolate of *N. veterana* had a resistance profile very similar to that of *N. nova* (amoxycillin MIC 2 mg/L, amoxycillin + clavulanate MIC 8 mg/L, ceftriaxone MIC >256 mg/L). One isolate of *N. asiatica* was equally sensitive to amoxycillin and amoxycillin + clavulanate (MIC 2 mg/L for both compounds), and was also sensitive to ceftriaxone (MIC 0.38 mg/L) and imipenem (MIC 0.25 mg/L).

The antibiotic susceptibilities of the 15 different *Nocardia* type strains are shown in Table 3. The susceptibility profiles to β-lactams were highly diverse for the different species. High-level resistance to β-lactams, including ceftriaxone and imipenem, was found in reference strains of *N. brasiliensis*, *N. otitidiscaviarum* and *N. niigatensis*. The reference strain of *N. pseudobrasiliensis* also displayed elevated MICs of amoxycillin and ceftriaxone but, unlike *N. brasiliensis*, remained susceptible to imipenem.

Fluoroquinolones

Resistance to ciprofloxacin was found in several *Nocardia* spp. including *N. nova*, *N. cyriacigeorgica*, *N. abscessus* and *N. brasiliensis*, while a variable susceptibility pattern was found in *N. farcinica*

Table 2. Frequency of susceptibility of various *Nocardia* spp. to selected antimicrobial agents

Species	Amoxycillin	Amoxycillin + clavulanate	Ceftriaxone	Imipenem	Ciprofloxacin	Clarithromycin	Tobramycin
Nocardia farcinica (*n* = 41)	10	100	22	100	54	5	7[b]
Nocardia nova (*n* = 20)	100	50[a]	70	100	0	100	60[b]
Nocardia cyriacigeorgica (*n* = 13)	62	83	100	100	0	0	100
Nocardia abscessus (*n* = 6)	100	100	100	50	0	67	100
Nocardia brasiliensis (*n* = 6)	33	100	50	0	0	0	100

[a]Higher MICs of amoxycillin + clavulanate than of amoxycillin alone for all *N. nova* isolates.
[b]Higher MICs of tobramycin for susceptible isolates of *N. farcinica* (MIC 2–4 mg/L) and for *N. nova* (MIC 1–4 mg/L) in comparison with all other *Nocardia* spp.

Table 3. MICs (mg/L) of culture collection reference strains of 15 *Nocardia* spp. to seven antimicrobial agents

Species (collection type/number)	Amoxycillin	Amoxycillin + clavulanate	Ceftriaxone	Imipenem	Ciprofloxacin	Clarithromycin	Tobramycin
Nocardia farcinica (DSM 43665^T)	4	1	8	0.19	0.38	4	24
Nocardia nova (DSM 43207^T)	0.25	4	6	0.032	> 32	< 0.016	8
Nocardia cyriacigeorgica (DSM 44484^T)	3	6	1.5	0.5	> 32	16	0.064
Nocardia brasiliensis (*NCTC 11294^T*)	> 256	0.5	> 256	> 32	32	> 256	< 0.016
Nocardia abscessus (DSM 44432^T)	0.25	0.25	0.38	1	12	3	0.25
Nocardia paucivorans (DSM 44836 ^{T})	0.25	2	0.5	0.064	0.012	> 256	< 0.016
Nocardia carnea (DSM 43397^T)	4	0.25	0.25	0.125	0.094	8	0.016
Nocardia asiatica (DSM 44668^T)	12	256	0.75	0.19	> 32	256	0.75
Nocardia niigatensis (DSM 44670^T)	> 256	128	32	> 32	1	0.032	0.094
Nocardia veterana (DSM 44445^T)	0.25	2	4	0.023	> 32	0.023	16
Nocardia africana (DSM44491^T)	4	32	32	0.125	> 32	0.016	48
Nocardia otitidiscaviarum (NCTC 1934^T)	> 256	64	> 256	> 32	1	> 256	1
Nocardia pseudobrasiliensis (DSM 44290^T)	> 256	3	256	2	0.25	0.016	1
Nocardia transvalensis (DSM 43405^T)	> 256	32	4	0.5	0.25	0.5	> 256
Nocardia asteroides (NCTC 11293^T)	4	16	2	0.38	4	2	< 0.016

(Tables 1 and 2). Among the less common species, *N. paucivorans* (two isolates), *N. carnea* (one isolate), and the type strains of these two species, were susceptible to ciprofloxacin.

Macrolides

Resistance to clarithromycin was observed in all isolates of *N. cyriacigeorgica* and *N. brasiliensis*, and in > 95% of *N. farcinica* isolates. Two strains of *N. asiatica* (one clinical isolate and one type strain) also displayed high-level resistance to clarithromycin (MIC > 256 mg/L). Conversely, *N. nova* isolates were uniformly susceptible to clarithromycin (MIC_{50} 0.016 mg/L; MIC_{90} 0.032 mg/L); two-thirds of the *N. abscessus* isolates were susceptible, but with MIC values close to the breakpoint limit (MIC_{50} 2 mg/L).

Aminoglycosides

All isolates of *Nocardia* spp. were susceptible to amikacin. Resistance to tobramycin was observed in > 90% of all *N. farcinica* isolates, and in 40% of the *N. nova* isolates. All susceptible isolates of *N. nova* and *N. farcinica* had elevated tobramycin MICs that were close to the breakpoint (MICs 1–4 mg/L). In contrast, most other species were highly susceptible to tobramycin (Tables 1 and 3), with the exception of *N. veterana* and *N. africana*, two species that are closely related taxonomically to *N. nova*. Furthermore, the reference strain of *N. transvalensis*, a species related closely to *N. asteroides sensu stricto* type IV, was also highly resistant to tobramycin (MIC > 256 mg/L), and had a moderate level of susceptibility to amikacin (MIC 8 mg/L) (Table 3).

DISCUSSION

Antimicrobial susceptibility testing of *Nocardia* isolates is recommended as a guide to therapy for cases of severe and disseminated infection, for refractory cases, or for patients who are intolerant to treatment with sulphonamides [1]. Testing may also be considered when relatively resistant species, or a newly described species, has been isolated, but laboratory results should always be interpreted with caution because of the paucity of studies that correlate in-vitro data with clinical outcome. Performance and interpretation of tests for antimicrobial susceptibility of *Nocardia* spp. are problematic because of the slow growth of these organisms. Furthermore, the frequent occurrence of clumps and aggregates makes the preparation of a uniform homogeneous inoculum difficult.

The CLSI have approved a standard method for susceptibility testing and interpretation by broth microdilution in cation-supplemented MH [16]. Other tests include disk-diffusion, agar dilution, Etests and the Bactec radiometric growth index method. Several studies have shown rates of inter- and intra-laboratory agreement and reproducibility of > 90% between these different methods and the approved broth microdilution standard method [17–19]. For example, a study testing 52 clinical isolates of *Nocardia* belonging to five different species by Etest and microbroth dilution MICs revealed 89% agreement for all drugs within ± 1 $\log_2$ dilution [18]. Using CLSI interpretative criteria, there was 96.2% agreement between Etests and microbroth dilution. In another study, Ambaye *et al*. [19] found 96.6% agreement between Etests and the consensus

susceptibility results of five different testing methods, with no major errors and only four very major errors (1.8% of the total tests) observed with Etests, all of which occurred with amoxycillin + clavulanate or ceftriaxone. Likewise, Tomlin *et al*. [20] reported >90% agreement between Etests and disk-diffusion results for 36 clinical isolates of *N. asteroides* using CLSI breakpoints for Enterobacteriaceae. Of the different methods, the Etest appears to be the most attractive method as it provides quantitative information (MIC values) that may be useful for tailoring therapy, and because it is highly flexible and suitable for the routine clinical laboratory.

There are few data in the literature concerning the antimicrobial susceptibility patterns of *Nocardia* isolates [15,18–22], and these early reports only included a small number of *Nocardia* isolates and suffered from a failure to identify isolates accurately to the species level. The present study determined the susceptibility patterns of 93 clinical isolates of *Nocardia* spp., all of which were identified by phenotypic and molecular tests [14]. As the isolates originated from a large number of laboratories and from different clinical sources, the distribution and prevalence of the various *Nocardia* spp. may be considered to be representative for Belgium.

N. farcinica, the predominant species in this study (45% of all clinical isolates) showed a rather typical multidrug resistance pattern, characterised by resistance to amoxycillin, ceftriaxone, clarithromycin and tobramycin, with susceptibility to amoxycillin + clavulanate and imipenem, in line with previous reports for this species [15,21].

N. nova, which accounted for *c*.20% of the clinical isolates, had a distinctive susceptibility pattern, characterised by susceptibility to amoxycillin and clarithromycin, but with resistance to amoxicillin + clavulanate in *c*.50% of the isolates, caused by the fact that *N. nova* isolates have an inducible membrane-bound penicillinase which is induced by clavulanic acid, but not by amoxycillin alone [22]. Indeed, positive β-lactamase activity was observed following induction by clavulanic acid in only six of the 20 *N. nova* isolates. A similar observation has been reported previously [19].

N. cyriacigeorgica accounts for >60% of nocardia infections in humans in the USA [1,2,], but ranked in only third position in the present study. All isolates of *N. cyriacigeorgica* were characterised by susceptibility to ceftriaxone and imipenem, and resistance to ciprofloxacin and clarithromycin. Variable results were observed with amoxycillin and amoxycillin + clavulanic acid. The susceptibility profile observed for the *N. cyriacigeorgica* isolates matched exactly with the type VI drug pattern of *N. asteroides* reported previously [15]. Thus, clinical isolates previously classified generically as *N. asteroides* with a type VI drug susceptibility pattern may have corresponded to *N. cyriacigeorgica*.

Among other species, some of the *N. abscessus* isolates had susceptibility patterns that were very similar to that reported previously for *N. asteroides sensu stricto* type I [15]. The results for the *N. brasiliensis* isolates mostly agreed with those of a recent study from Mexico [23]. However, few conclusions can be reached concerning the drug resistance profiles of the less common species of *Nocardia* since they were represented by only a few isolates. Some of these species (e.g., *N. paucivorans* and *N. carnea*) have been reported only rarely as pathogens in humans [1,10], while others (*N. asiatica*, *N. niigatensis*) were only described quite recently [11,12].

Wide variations in the resistance profiles to β-lactams were observed in both clinical isolates and in type strains. Several distinct β-lactamases, inducible or expressed constitutively, have been characterised in various *Nocardia* spp. including *N. asteroides sensu stricto* [24] and *N. farcinica* [25,26], and it is likely that these account, at least in part, for the variations in resistance to β-lactams among species. β-Lactamases of *Nocardia* have been shown to differ in their spectrum and kinetics towards different substrates. All characterised *Nocardia* β-lactamases are class A β-lactamases, which behave as penicillinases and confer high levels of resistance to amoxycillin, piperacillin and cephalothin [24–26]. Their hydrolytic activity is inhibited partially by clavulanic acid, but almost not at all by sulbactam and tazobactam, which probably explains the lack of activity of piperacillin + tazobactam against most *Nocardia* isolates. Resistance to ceftriaxone and to imipenem does not seem to be mediated by β-lactamases, but rather by decreased affinities of penicillin-binding proteins for these molecules [26].

The present study also confirmed the excellent in-vitro activity of trimethoprim + sulphamethoxazole, amikacin and linezolid against all *Nocardia* spp. [18,19,22,23,27]. Although no resistance to

minocycline was observed, the MIC values fell in the decreased susceptibility category for most isolates, as has been reported previously [15,20].

In conclusion, the present study detected large variations in the antimicrobial susceptibility profiles of different *Nocardia* spp. and the existence of homogeneous drug resistance patterns among several species included formerly in the *N. asteroides* complex [15,19,20]. Susceptibility testing of selected antimicrobial agents by Etest could be useful, both for therapeutic purposes and as a simple complementary tool for identification of several of the *Nocardia* spp. associated frequently with human infections. However, >90% of all clinical *Nocardia* isolates encountered in this survey belonged to only five species; the small number of strains for some species and the limited geographical area in which the study was performed do not permit extrapolation of the results to other areas. Further studies with a larger panel of isolates from more diverse geographical regions, including other *Nocardia* spp. occurring less commonly in humans, are required to validate and extend the results.

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