



MOLECULAR IDENTIFICATION AND SUSCEPTIBILITIES OF NOCARDIA CLINICAL STRAINS

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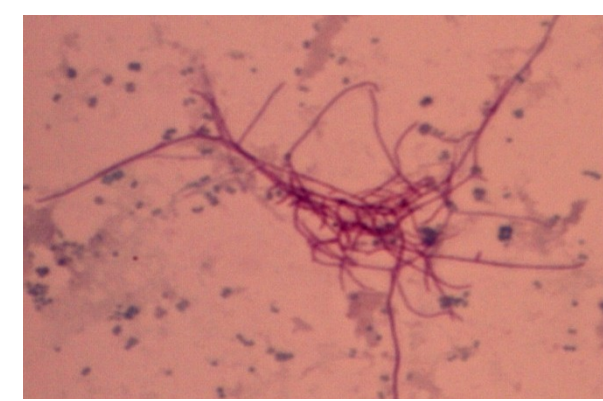
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Background

Species of the genus *Nocardia* are opportunistic pathogens that cause pulmonary, disseminated or subcutaneous infections among immunocompromised or previously healthy individuals. There are currently 86 recognized *Nocardia* spp. more than half of which have been described during the last 10 years; most have been isolated from clinical specimens (1,2). Trimethoprim-sulfamethoxazole (TMP-SXM) has long been considered the therapeutic agent of choice for monotherapy while combinations of TMP-SXM with amikacin, carbapenems, or ceftriaxone are often used for severe systemic infections(2). Over the last years there has been an increasing interest in the taxonomy and susceptibility patterns of clinical isolates.



Modified Kinyoun stain of *Nocardia* strain from a pus specimen (Fig 1) and from culture in MGIT960 tube (Fig 2)(Mx600)

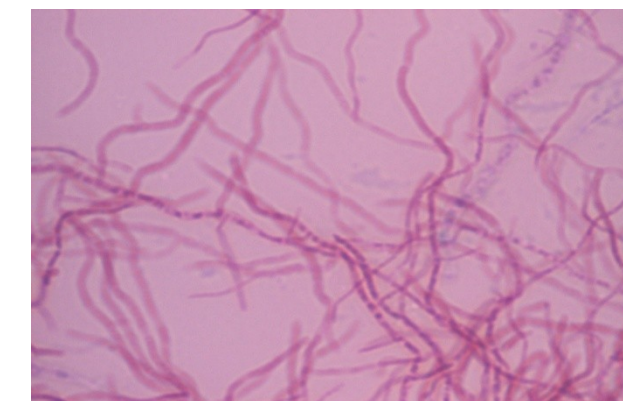


Fig 1

Fig 2

Objective

The present study pursued molecular identification and defined antimicrobial susceptibility of *Nocardia* species strains isolated or submitted for testing at a 700-bed tertiary health care center in Athens during a four year period.

Materials & Methods

All consecutive isolates (33 strains) obtained during 2010-2013 and belonging to *Nocardia* species as per phenotypic testing were included in the study.

Twenty-two strains were isolated at Attikon University Hospital.

Additionally, 11 strains were submitted for identification from other hospitals, mostly in Athens.

Definitive identification was determined by PCR-RFLP analysis of the hsp65 gene (440 bp) as previously described [3].

Additionally, sequencing of the 16S rDNA gene (600 bp) represented the reference identification method for selected isolates; it was performed using the protocols and primers previously described [4]. The sequence was aligned with the sequences of GenBank (www.ncbi.nlm.nih.gov) database. Isolates with sequence similarities of 97-99.5% to references were identified as *Nocardia* sp. and were considered that they likely belong to yet undescribed taxa.

Antimicrobial Susceptibility Testing (AST) was performed by standard CLSI broth microdilution method (5) using commercial broth microdilution panels (RAPMYCOI, TREK Diagnostic systems). MICs were determined for Amikacin (AMK), Linezolid (LZD), TMP-SXM (1:19), Ceftriaxone (AXO), Tobramycin (TOB), Cefepime (FEP), Imipenem (IMI), Moxifloxacin(MXF), Clarithromycin (CLA), Ciprofloxacin(CIP), Doxycycline(DOX), Minocycline(MIN) and Amoxicillin-Clavulanic acid (1:2)(AUG) and interpreted as recommended by CLSI (Table 1,5).

Clinical data were recorded when available.

Results

Among 33 unique *Nocardia* isolates

the majority were recovered from sputum (28) followed by pus (4) and peripheral blood (1) cultures (Table 2).

Ten species were identified: *N. cyriacigeorgica* (12 strains; 36.4%), *N. nova* (4), *N. carnea* (3), *N. abscessus* (3), *N. brasiliensis* (2), *N. farcinica* (2), *N. flavorosea* (1), *N. otitidiscaviarum* (1), *N. veterana* (1), and *N. paucivorans* (1) (Table 2).

3 *Nocardia* isolates remained unidentified (Table 3, Fig 3)

Nocardia sp. GR-381 is closely related to *N.mexicana*.

Nocardia sp. GR-8491 is closely related to *N.carnea*

Nocardia sp. GR-8271 is closely related to *N.flavorosea*

Antimicrobial Agent	Minimal Inhibitory Concentration (µg/ml) for category			Range of Concentrations (µg/ml)	Number of Concentrations
	S	I	R		
Primary					
AMK	≤8		≥16	1-64	7
AUG	≤8/4	16/8	≥ 32/16	2/1-64/32	6
AXO	≤8	16-32	≥64	4-64	5
CIP	≤1	2	≥4	0.12-4	6
CLA	≤2	4	≥8	0.06-16	9
IMI	≤4	8	≥16	2-64	6
LZD	≤8			1-32	6
MIN	≤1	2-4	≥8	1-8	4
MXF	≤1	2	≥4	0.25-8	6
SXT	2/38		4/76	0.25/4.75-8/152	6
TOB	≤8	16	≥32	1-16	5
Secondary					
AMK	≤8		≥16	1-64	7
AUG	≤8/4	16/8	≥ 32/16	2/1-64/32	6
AXO	≤8	16-32	≥64	4-64	5
CIP	≤1	2	≥4	0.12-4	6
CLA	≤2	4	≥8	0.06-16	9
IMI	≤4	8	≥16	2-64	6
LZD	≤8			1-32	6
MIN	≤1	2-4	≥8	1-8	4
MXF	≤1	2	≥4	0.25-8	6
SXT	2/38		4/76	0.25/4.75-8/152	6
TOB	≤8	16	≥32	1-16	5

Table 1. Broth microdilution Interpretation criteria for *Nocardia* as suggested by CLSI (5), and range and number of the respective antibiotic concentrations tested by the microdilution panels RAPMYCOI, SXT, and TOB.

Identification	No of strains	Type of specimen
<i>N. cyriacigeorgica</i>	10	Sputum
<i>N. cyriacigeorgica</i>	1	BAL
<i>N. cyriacigeorgica</i>	1	Lung abscess
<i>N. abscessus</i>	3	Sputum
<i>N. carnea</i>	3	Sputum
<i>N. nova</i>	3	Sputum
<i>N. nova</i>	1	Pus
<i>N. brasiliensis</i>	2	Pus
<i>N. veterana</i>	1	Blood culture
<i>N. farcinica</i>	1	Sputum
<i>N. farcinica</i>	1	Pus
<i>N. flavorosea</i>	1	Sputum
<i>N. paucivorans</i>	1	Sputum
<i>N. otitidiscaviarum</i>	1	Sputum
<i>Nocardia</i> sp. GR-381	1	BAL
<i>Nocardia</i> sp. GR-8275	1	Sputum
<i>Nocardia</i> sp. GR-8491	1	Sputum

Table 2. Distribution of *Nocardia* species isolated from clinical specimens.

Strain	Type strain	Identity
<i>Nocardia</i> sp.GR-381	<i>N. mexicana</i> DSM44952 (JF797310)	584/600 (97.3%)
	<i>N.crassostreae</i> ATCC700418 (AY756548)	582/598 (97.3%)
	<i>N. nova</i> DSM43256 (AF430031)	583/600 (97.2%)
<i>Nocardia</i> sp.GR-8275	<i>N. flavorosea</i> JCM3332 (AY756552)	536/539 (99.4%)
	<i>N.sienata</i> E244810 (GQ905494)	535/539 (99.3%)
	<i>N. rhamnosiphila</i> X0490 (KC905494)	534/539 (99.1%)
	<i>N. carnea</i> ATCC6847 (JF797307)	586/589 (99.5%)
<i>Nocardia</i> sp.GR-8491	<i>N. rhamnosiphila</i> X0490 (KC905494)	583/589 (99%)
	<i>N. flavorosea</i> JCM3332 (AY756552)	583/589 (99%)

Table 3. 16S rDNA sequence comparison results for the three unidentified *Nocardia* strains

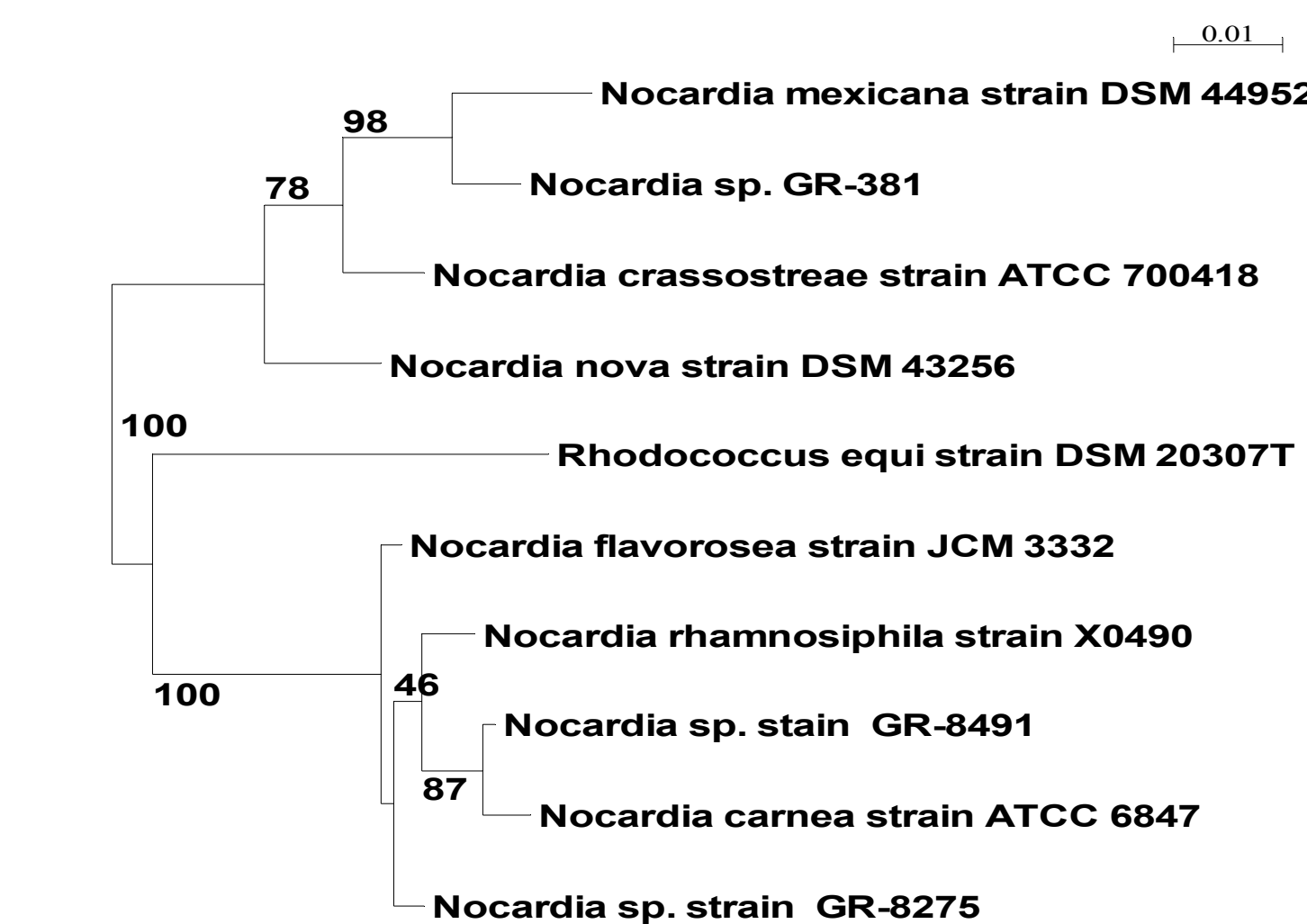


Fig 5. Phylogenetic tree of 16S rDNA sequences of the three unidentified nocardial isolates and type strains. The tree was created using the neighbour-joining method. Numbers on the tree indicate bootstrap values for the branch points. The tree was rooted with the use of *Rhodococcus equi* strain DSM203077 as the outgroup. The scale bar represents a 1% difference in nucleotide sequences.

Results

Clinical data were available for 26 patients.

All of them presented with immunosuppression and isolation was considered clinically important.

Notably, the uncommon species isolate of *N. veterana* was isolated from a blood culture.

Species (no of isolates)	AUG	AXO	IMI	CIP	MIN	LZD	TMP-SMX	TOB	AMK	CLA
<i>N. cyriacigeorgica</i> (12) expected	R	9S 3I S	9R 3S S	R	2S 9I 1R V	S	S	S	S	R
<i>N. nova</i> (4) expected	R	S	S	R	I	S	S	S	R	S
<i>N. abscessus</i> (3) expected	S	S	R	R	I	S	S	S	S	R
<i>N. veterana</i> (1) expected	R	I	S	R	I	S	S	R	S	R
<i>N. brasiliensis</i> (2) expected	S	R	R	R	I	S	S	S	S	R
<i>N. farcinica</i> (2) expected	S	R	R	S	I	S	S	R	S	R
<i>N. otitidiscaviarum</i> (1) expected	R	R	R	R	S	S	S	S	S	R
<i>N. paucivorans</i> (1)	I	S	S	S	S	S	S	S	S	I
<i>Nocardia</i> sp. GR-381	R	S	R	R	I	S	S	R	R	R
<i>N. carnea</i> (2)	I,R	S	S	S	I	S	R	S	S	S
<i>Nocardia</i> sp. GR-8491	I	S	I	S	S	S	S	S	S	S
<i>N. flavorosea</i> (1)	R	S	S	S	S	S	S	S	S	I
<i>Nocardia</i> sp. GR-8275	R	S	S	S	S	S	R	S	S	R

Table 4. Antimicrobial susceptibility pattern of *Nocardia* isolates in comparison with the expected patterns as suggested by CLSI (6)

	MIC ₅₀ (µg/ml)	MIC ₉₀ (µg/ml)	range (µg/ml)	Number of susceptible strains (%)
LZD	2	4	<1-4	33 (100)
AMK	<1	<1	<1-32	32 (97)
TMP-SMX	1/19	4/76	<0.25/4.75-8/152	30 (91)
AXO	<4	16	<4- >64	23 (69.6)
TOB	<1	32	<1 - >16	24 (72.7)
FEP	8	32	<1- >32	17 (51.5)
IMI	8	32	<2- >64	13 (39.3)
MXF	2	4	<0.25-8	12 (36.4)
CLA	8	>16	<0.06- >16	7 (21.2)
CIP	>4	>4	<0.12- >4	8 (24.2)
DOX	2	16	<0.12-16	9 (27.3)
MIN	4	4	<1-8	7 (21.2)
AUG	32/16	>64/32	<2/1- >64/32	5 (15.2)

Table 5. Susceptibilities of the *Nocardia* isolates to 13 antibiotics.

	TMP-SMX		Imipenem		Ceftriaxone		Amikacin	
	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀
<i>N. cyriacigeorgica</i> (n=12)	1/19	2/38	16	32	8	16	<1	<1
<i>N. nova</i> (n=4)	1/19	1/19	<2	<2	8	8	<1	<1
<i>N. abscessus</i> (n=3)	1/19	1/19	8	16	<4	<4	<1	<1

Table 6. MIC₅₀ and MIC₉₀ values for TMP-SMX, Imipenem, Ceftriaxone and Amikacin among the most common species.

Results

In total, susceptibility testing showed:

>100% susceptibility to linezolid

>97% susceptibility to amikacin

> 91% trimethoprim-sulfamethoxazole and

>69.6% susceptibility to ceftriaxone

An unusually high imipenem resistance rate (75%) was detected among isolates of *N. cyriacigeorgica*

Resistance to trimethoprim-sulfamethoxazole was restricted to the species *N. carnea* (MIC range: 4/76- 8/152)

Also the unidentified strain *Nocardia* sp GR-8271 was resistant to trimethoprim-sulfamethoxazole (MIC:8/152)

Conclusion

This report on *Nocardia* spp. isolation reveals the following:
despite the limited number of strains, a broad spectrum of species was identified
N. cyriacigeorgica represented the most common pathogen.
Species diversity and species-associated resistance indicate the necessity of routine molecular identification as well as antimicrobial susceptibility testing in order to treat nocardial infections efficiently

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