Spectrum and clinicomicro-biological profile of *Nocardia* keratitis caused by rare species of *Nocardia* identified by 16S rRNA gene sequencing

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Abstract

Purpose To report the predisposing factors, clinical profile, and treatment outcome of patients with keratitis that was caused by a rare species of *Nocardia*.

Methods Between April 2006 and October 2008, medical and microbiology records of patients with keratitis that was caused by *Nocardia* were reviewed. Isolates were identified to species level by 16S rRNA gene sequencing, and antibiotic susceptibility testing was performed by E-test to amikacin, azithromycin, clarithromycin, ciprofloxacin, gatifloxacin, and tobramycin.

Results Of 19 cases with *Nocardia* keratitis, 8 were caused by unusual isolates. Species distribution among the eight isolates was as follows: the *Nocardia levis* (2 of 8), *Nocardia amamiensis* (2 of 8), *Nocardia abscessus* (1 of 8), *Nocardia puris* (1 of 8), *Nocardia beijingensis* (1 of 8), and *Nocardia thailandica* (1 of 8). All eight (100%) isolates were sensitive to amikacin and tobramycin. Trauma to the cornea was the major predisposing factor in seven of the eight patients. Five patients presented with the characteristic wreath-pattern infiltrate. The infection resolved to topical therapy with amikacin sulphate (2.5%) in six patients, whereas two patients treated with the same antibiotic were lost to follow-up at a point when the lesion was showing signs of resolution.

Conclusion The healing response in *Nocardia* keratitis cases was the same, irrespective of species.

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Keywords: keratitis; *N. levis*; *N. amamiensis*; *N. thailandica*; amikacin

Introduction

Organisms of the genus *Nocardia* are a rare cause of infectious keratitis.¹ Various species described to cause keratitis include *Nocardia asteroides*, *Nocardia arthritidis*, *Nocardia pseudobrasiliensis*, *Nocardia gypsoides*, *Nocardia farcinica*, *Nocardia caviae*, *Nocardia cyriacigeorgica*, and *Nocardia otitidiscaviarum*.^{1,2} In this study, we report the predisposing factors, clinical profile, and treatment outcome of keratitis caused by other rare species of *Nocardia*.

Methods

We reviewed microbiology records of patients with keratitis examined at the L. V. Prasad Eye Institute (Hyderabad, India) between April 2006 and October 2008. Out of 4918 suspected cases of microbial keratitis, 2001 were culture proven. Of the 2001 culture-proven cases, the growth of *Nocardia* was observed in 19 (0.9%) cases during the study period. All 19 isolates were subjected to identification by gene sequencing of 16S rRNA. Review of the published literature in English using PubMed showed that eight of these isolates that were identified by gene sequencing have never been reported.

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We conducted a retrospective review of medical records of all these cases.

All patients underwent a detailed clinical evaluation. They were subjected to corneal scrapings, and the material was subjected to standard microbiology procedures as described previously.³

Genetic identification was performed by amplifying a 606-bp fragment of the 16S rRNA gene of *Nocardia* as described by Rodriguez-Nava *et al*⁴. Sequencing was performed with fluorescence-labelled dideoxynucleotide terminators using an ABI 3130 XL automated sequencer, following the manufacturer's instructions (PE Applied Biosystems, Foster, CA, USA). Sequences were analyzed and identified using the MEGABLAST search program of GenBank databases and Bibi software (<http://pbil.univ-lyon1.fr/bibi>). An isolate was assigned to a particular species if the 16S rRNA sequence of the isolate was >99% similar to the type strain of the most closely related species, indicated by both NCBI BLAST and Bibi database programs. *In vitro* antibiotic susceptibility was determined using the E-test (AB Biodisk, Solna, Sweden), and test results were interpreted according to the CLSI guidelines (approved standards M24-A: CLSI 2009).

The Institutional Review Board of the L.V. Prasad Eye Institute (Hyderabad, India) approved this study.

Results

Species of *Nocardia*, their percentage similarity with known strains deposited in the GenBank, and *in vitro* antimicrobial susceptibility results are shown in Table 1. Minimum inhibitory concentration (MIC)₉₀ values were 0.75 µg/ml for amikacin, 1 µg/ml for tobramycin, 08 µg/ml for both ciprofloxacin and gatifloxacin, and 16 µg/ml for both azithromycin and clarithromycin. Predisposing factors, clinical picture, and treatment outcome of all the eight patients are shown in Table 2. All patients presented with complaints of pain, redness, and watering from 4 days to 2 months duration. Trauma was the major predisposing factor in seven of the eight

patients. Five patients presented with the characteristic wreath-pattern infiltrate. All patients were treated with topical amikacin sulphate (2.5%). The infection resolved in six patients, whereas two patients were lost to follow-up at a point when the lesion was showing signs of resolution.

Discussion

Analysis of gene sequences has contributed to our understanding of the phylogenetic relationships of *Nocardia* and has resulted in the recognition of numerous new species.⁵ Various molecular methods used for the identification include gene sequencing of 16S rRNA and *hsp65*, pyrosequencing of 16S rRNA and ribotyping.^{2,5} Although Yin *et al*² reported *hsp65* gene sequencing as the more reliable method, there is no consensus on the superiority of one over the other.

To the best of our knowledge, this is the first report describing the predisposing factors, clinical picture, and treatment outcome of microbial keratitis caused by *Nocardia levis*, *Nocardia puris*, *Nocardia thailandica*, and *Nocardia amamiensis*. Although *N. levis* and *N. amamiensis* were not isolated from human infections,^{6,7} *N. puris* and *N. thailandica* were isolated from patients with abscess and choroiditis.^{8,9}

Trauma to the cornea was a major predisposing factor in this study, a finding similar to that of Lalitha *et al*¹⁰. Five of these eight patients presented with characteristic patchy anterior stromal infiltrates surrounded by yellow white discrete pinhead-sized lesions arranged in a wreath pattern, similar to that described for other reported species.^{1,10}

Although Lalitha *et al*¹⁰ reported that the wreath pattern is more commonly seen in patients presenting within weeks of the onset of symptoms, two patients in our series had this clinical picture in spite of delayed presentation (patient nos 2 and 4).

All the newly identified isolates in this series were sensitive to amikacin and tobramycin, but MIC₉₀ values

Table 1 Species of *Nocardia* and their susceptibility to different antibiotics

Patient no.	Species	Reference sequence accession number ^a	% Similarity	AK	AZ	CM	CP	GF	TB
1	<i>Nocardia puris</i>	AY903618	100	S	S	R	R	R	S
2	<i>Nocardia beijingensis</i>	AB094650	100	S	R	R	S	S	S
3	<i>Nocardia levis</i>	AM746988	99.9	S	R	R	R	R	S
4	<i>Nocardia thailandica</i>	AB126874	100	S	R	R	R	R	S
5	<i>Nocardia amamiensis</i>	AB275164	99.7	S	R	R	R	R	S
6	<i>Nocardia amamiensis</i>	AB275164	99.9	S	R	R	S	S	S
7	<i>Nocardia levis</i>	AM746988	100	S	S	S	S	S	S
8	<i>Nocardia abscessus</i>	AY544980	100	S	R	R	R	R	S

Abbreviations: AK, amikacin; AZ, azithromycin; CM, clarithromycin; CP, ciprofloxacin; GF, gatifloxacin; TB, tobramycin; S, sensitive; R, resistant.

^aGenBank sequence to which similarity was observed.

Table 2 Predisposing factors, clinical picture, and treatment outcome of patients with *Nocardia* keratitis

Patient no.	Age and sex	Duration of symptoms	Predisposing factor	Initial VA	Species	Clinical picture	Treatment and duration	Outcome and final VA
1	65 M	1 Month	Injury with wooden piece	20/400	<i>Nocardia puris</i>	Diffuse infiltrate, Endoexudate	Amikacin (topical) and cotrimoxazole (topical and oral) 6 weeks	Resolved, 20/80
2	30 M	2 Months	Injury with stone	20/80	<i>Nocardia beijingensis</i>	Wreath pattern	Amikacin (topical) 3 weeks	Resolved, 20/30
3	35 M	2 Weeks	Fall of dust	CF 50 m	<i>Nocardia levis</i>	Wreath pattern	Amikacin (topical) 2 weeks	20/50 Resolved
4	22 M	1 Month	Injury while working in field	20/60	<i>Nocardia thailandica</i>	Wreath pattern	Cefazolin (topical) and ciprofloxacin (topical) 2 weeks	Lost to follow-up, 20/50
5	20 M	2 Months	Injury with stone	20/200	<i>Nocardia amamiensis</i>	Corneal infiltrate with vascularization	Amikacin (topical) and cefazolin (topical) 4 weeks	Resolved, 20/125
6	45 M	20 Days	Injury with sand	HM	<i>Nocardia amamiensis</i>	Wreath pattern	Amikacin (topical) and cotrimoxazole (oral) 6 weeks	Resolved, counting fingers 1 m
7	55 M	8 Days	Not known	20/125	<i>Nocardia levis</i>	Wreath pattern	Amikacin (topical), 1 week	Lost to follow-up, 20/40
8	48 F	20 Days	Injury with grass	20/120	<i>Nocardia abscessus</i>	Peripheral infiltrate	Amikacin (topical) and cotrimoxazole (oral) 5 weeks	Resolved 20/50

Abbreviations: CF, counting finger; HM, hand movement; VA, visual acuity.

of tobramycin were higher than those of amikacin. In a recent report, Pandya and Petsoglou¹¹ reported a case of amikacin resistant *Nocardia transvalensis* keratitis. Similarly, Eggnik *et al*¹² reported increased morbidity in keratitis due to *N. farcinica* because of its inherent resistance to antibiotics. In this series, the infection in all patients responded favourably to amikacin sulphate (2.5%) therapy, a finding similar to that reported by Lalitha *et al*¹⁰

Conclusions

Keratitis in humans can be caused by various *Nocardia* species. The healing response in *Nocardia* keratitis cases was the same, irrespective of species.

Summary

What was known before

- *Nocardia* species described to cause keratitis include *Nocardia asteroides*, *Nocardia arthritidis*, *Nocardia pseudobrasiliensis*, *Nocardia gypsoides*, *Nocardia farcinica*, *Nocardia caviae*, *Nocardia cyriacigeorgica*, and *Nocardia otitidiscaviarum*.

What this study adds

- This is the first report describing the predisposing factors, clinical picture, and treatment outcome of the microbial keratitis caused by *Nocardia levis*, *Nocardia puris*, *Nocardia thailandica*, and *Nocardia amamiensis*.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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