Enfermedades Infecciosas y Microbiología Clínica 40 (2022) 562–567

Enfermedades Infecciosas y Microbiología Clínica

www.elsevier.es/eimc

Brief report

Epidemiology and *in vitro* antimicrobial susceptibility of aerobic Actinomycetales in a clinical setting

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ARTICLE INFO

Article history:
Received 28 December 2020
Accepted 22 February 2021

Keywords:
Actinomycetes
Nocardia
Tsukamurella
Gordonia
MALDI-TOF
Antimicrobial susceptibility

ABSTRACT

Introduction: The incidence of infections caused by aerobic actinomycetes is increasing. Recent changes in taxonomy and the variability in susceptibility patterns among species make necessary a proper identification and antibiotic susceptibility testing.
Material and methods: Fifty-three strains of aerobic actinomycetes were identified by MALDI-TOF MS using the VITEK MS *Mycobacterium/Nocardia* kit (bioMérieux, France) in a tertiary hospital in Spain during a six-year period. Antimicrobial susceptibility testing of the isolates was performed using the Sensititre Rapmycoi microdilution panel (Thermo Fisher Scientific, Massachusetts, USA).
Results: Forty strains of *Nocardia* spp. were identified in the study, being *N. farcinica* and *N. cyriacigeorgica* the most prevalent ones. All isolates were susceptible to linezolid and the resistance to amikacin was only observed in one isolate of *Gordonia sputi*. Resistance to cotrimoxazole was only found in five isolates.
Conclusions: Routine identification and antimicrobial susceptibility testing of aerobic actinomycetes is advisable for an efficient identification of species and effective treatment.

Epidemiología y sensibilidad *in vitro* de especies de Actinomycetales aerobios en la rutina asistencial

Palabras clave:
Actinomycetales
Nocardia
Tsukamurella
Gordonia
MALDI-TOF
Sensibilidad antimicrobiana

RESUMEN

Introducción: La incidencia de infecciones por actinomicetos aerobios está aumentando. Los recientes cambios en la taxonomía y la variabilidad en la sensibilidad entre especies hacen necesaria una identificación y estudio de sensibilidad adecuados.
Material y métodos: Se identificaron 53 cepas de actinomicetos aerobios mediante MALDI-TOF utilizando el *kit* VITEK-MS *Mycobacterium/Nocardia* (bioMérieux, Francia) en un hospital terciario español durante seis años. Los estudios de sensibilidad de los aislados se realizaron utilizando el panel de microdilución Sensititre Rapmycoi (Thermo Fisher Scientific, Massachusetts, EE. UU.).
Resultados: Se identificaron 40 cepas de *Nocardia* spp., siendo *Nocardia farcinica* y *Nocardia cyriacigeorgica* las más prevalentes. Todos los aislados fueron sensibles a linezolid, y solo se detectó resistencia a amikacina en un aislado de *Gordonia sputi*. Solo se encontró resistencia al cotrimoxazol en cinco aislados.
Conclusiones: Es aconsejable realizar la identificación de rutina y las pruebas de sensibilidad antimicrobiana de los actinomicetos aerobios para conseguir una identificación eficiente de las especies y un tratamiento eficaz.

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https://doi.org/10.1016/j.eimce.2021.02.014
2529-993X /

Introduction

The incidence of infections caused by aerobic actinomycetes (especially *Nocardia* spp.) has increased in the last years, in association with the improvement in detection and diagnostic techniques, and the higher prevalence of immunosuppressed patients.[1] Usually, nocardiosis appears as pulmonary disease, although the infection could disseminate from the lungs, and local abscesses may result from direct inoculation.[1,2] The most typical sites for dissemination are the central nervous system, skin and subcutaneous tissue, and other organs.[1–4] Optimal treatment regimens for nocardiosis have not been established, but recommended ones use antibiotics with a proven efficacy in clinical studies.[5–7] However, it is recommended that antimicrobial *in vitro* testing should be performed to assist treatment management.[1,2,4]

Other species of actinomycetes that may cause infections in humans belong to the genus *Rhodococcus*, *Gordonia* and *Tsukamurella*.[1] It is important to individualize the treatment of those infections according to *in vitro* susceptibility results because of the lack of clinical data. The difficulties with the conventional identification methods using biochemical tests and the current molecular taxonomy as gold standard for the identification, by using PCR and sequencing, have led us to use matrix assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry as a routine method for the identification of these species.

Given the wide spectrum of clinical manifestations, the increasing number of species of aerobic actinomycetes, the different prevalence and their variation in susceptibility to antibiotics, it is necessary an accurate identification and susceptibility testing for an efficient and effective treatment.[2,8,9]

Materials and methods

All isolates of aerobic actinomycetes that were recovered in our hospital from different clinical samples between 2014 and July 2020 were included.

Identification

Strains were grown on tryptic-soy agar plates supplemented with 5% of sheep blood (bioMérieux, Marcy l'Étoile, France) and incubated for 48–72 h at 37 °C. Species identification was performed by MALDI-TOF mass spectrometry, using the VITEK MS® *Mycobacterium/Nocardia* kit (bioMérieux, Marcy l'Étoile, France). This kit provides some reagents and consumables needed to process samples by inactivation and protein extraction. Briefly, samples were collected from the plate and re-suspended in 500 μL of 70% ethanol in glass beads vials. They were vortexed for 15 min and incubated at room temperature for 10 min. The supernatant was transferred into new tubes and centrifuged at 13,000×*g* for 2 min. The pellet was resuspended in 10 μL of 70% formic acid and subsequently in 10 μL of acetonitrile. Samples were vortexed and centrifuged at 13,000×*g* for 2 min. One μL of the supernatant was used for MALDI-TOF testing. Two different data bases (bioMérieux, Marcy l'Étoile, France) were used for the identification at species level, IVD® v3.2 (close database for clinical practice) and Saramis® v.15/RUO (open database for research use). Some of the isolates randomly selected were submitted to the National Center of Microbiology (Majadahonda, Spain) for 16S rRNA gene sequencing to verify the correct identification at species level given by MALDI-TOF.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was tested by the broth microdilution method using Sensititre® RAPMYCOI microtiter plates (ThermoFisher Scientific, Massachusetts, USA), according to the reference methodology for aerobic actinomycetes.[10] Tested antibiotics were amikacin, amoxicilin-clavulanate, cefoxitin, ceftriaxone, ciprofloxacin, clarithromycin, doxycycline, imipenem, linezolid, minocycline, moxifloxacin, tigecycline, tobramycin, and cotrimoxazole. Minimum inhibitory concentration (MIC) was determined after 72 h of incubation at 37 °C and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[10] Tigecycline breakpoints for *Enterobacteriaceae* were used for interpretation. *Staphylococcus aureus* ATCC 29213 was used as quality control.

Results

During the study period a total of 53 non-duplicated actinomycetes isolates were studied, including *Nocardia* spp. (40/53), *Gordonia* spp. (10/53), and *Tsukamurella* spp. (3/53) from 54 patients. One patient had two different isolates of *Nocardia* spp.

Among *Nocardia* spp. isolates, *N. farcinica* (12) and *N. cyriacigeorgica* (11) were the most prevalent species, followed by *N. nova/africanum* (9), *N. paucivorans* (2), *N. abscessus* (1), *N. carnea* (1), *N. neocaledonensis* (1), *N. otidiscaviarum* (1), *N. veterana* (1) and *N. wallacei* (1). Within the other aerobic actinomycetes the isolated species were *G. sputi* (6), *G. bronchialis* (4), *T. pulmonis* (2) and *T. tyrosinosolvens* (1). No discrepancies were found between the results obtained by MALDI-TOF and 16S rRNA on the studied isolates.

Most of the isolates were recovered from the respiratory tract (80%) including 32 isolates of *Nocardia* spp., seven of *Gordonia* spp., and one of *Tsukamurella* spp.; followed by subcutaneous abscesses and wounds (10.9%) including six *Nocardia* spp. isolates and one *Gordonia* spp. isolate; and blood (9.1%) including two *Gordonia* spp., one *Nocardia* spp., and one *Tsukamurella* spp. *N. cyriacigeorgica* was the most frequent species isolated from the respiratory tract (27.5%; 11/40), followed by *N. nova/africanum* (22.5%, 9/40). *N. farcinica* was the predominant species from skin and soft tissues samples (42.9%; 3/7), and *G. sputi* from blood samples (50%; 2/4).

Antimicrobial susceptibility of *Nocardia* spp. to the 14 antibiotics tested is summarized in Table 1. All the isolates were susceptible to amikacin and linezolid, and most of them were also susceptible to cotrimoxazole (97.5%), followed by imipenem (67.5%), tigecycline (52.5%) and tobramycin (52.5%).

Results of antimicrobial susceptibility testing for *Gordonia* spp. and *Tsukamurella* spp. isolates are shown in Table 2. All the strains were susceptible to linezolid, and most of them were susceptible to amikacin, ciprofloxacin, and moxifloxacin.

Discussion

Because of the recent *Nocardia* species discovered during the past years,[2] and the diversity of susceptibility patterns, it is necessary to have available techniques that could give a rapid and accurate identification at the species level.[11,12] In this scenario, MALDI-TOF may represent a quick and reliable option, as it is seen in previous studies.[12,13] It has been demonstrated that this tool is also potentially useful for identifying species of the genera *Gordonia*[14] and *Tsukamurella*.[15] A proper and rapid identification could be useful to guide antibiotic therapy, until *in vitro* susceptibility results are available. However, MALTI-TOF shows some limitations, as uncommon species may be still difficult to identify depending on the

Table 1
MIC_{50}, MIC_{90}, and range (mg/L), and percentage of susceptible isolates of *Nocardia* spp.

Antimicrobial agent	Breakpoints	Species (no. of strains tested)				
		All *Nocardia* spp. (*n* = 40)	*N. farcinica* (*n* = 12)	*N. cyriacigeorgica* (*n* = 11)	*N. nova/africanum* (*n* = 9)	Other *Nocardia* spp. (*n* = 8)
Amikacin	S ≤ 8					
Susceptible [*n* (%)]		40 (100)	12 (100)	11 (100)	9 (100)	8 (100)
MIC range		<1 to 4	<1 to 2	<1	<1	<1 to 4
MIC_{50}		<1	<1	<1	<1	<1
MIC_{90}		2	2	<1	<1	4
Amoxicillin-clavulanate	S ≤ 8					
Susceptible [*n* (%)]		12 (30)	5 (41.7)	2 (18.2)	2 (22.2)	3 (37.5)
MIC range		<2 to >64	<2 to >64	<2 to >64	8 to >64	<1 to >64
MIC_{50}		32	32	16	32	32
MIC_{90}		>64	>64	>64	>64	>64
Cefoxitin	S ≤ 16					
Susceptible [*n* (%)]		9 (22.5)	1 (8.3)	3 (27.3)	1 (11.1)	4 (50)
MIC range		<4 to >128	8– 128	<4 to 128	16 to >64	8 to >128
MIC_{50}		64	64	32	64	16
MIC_{90}		>128	>128	128	>128	>128
Ceftriaxone	S ≤ 8					
Susceptible [*n* (%)]		24 (60)	3 (25)	10 (90.1)	4 (44.4)	7 (87.5)
MIC range		<4 to >64	<4 to >64	<4 to 16	<4 to >64	<4 to >64
MIC_{50}		8	8	<4	8	<4
MIC_{90}		>64	>64	8	>64	>64
Ciprofloxacin	S ≤ 1					
Susceptible [*n* (%)]		12 (30)	8 (66.7)	0	0	5 (62.5)
MIC range		<0.12 to >4	<0.12 to >4	2 to >4	>4	0.25 to >4
MIC_{50}		4	2	>4	>4	0.5
MIC_{90}		>4	>4	>4	>4	>4
Clarithromycin	S ≤ 2					
Susceptible [*n* (%)]		21 (52.5)	3 (25)	6 (54.5)	8 (88.9)	4 (0.5)
MIC range		0.25 to >16	0.5 to >16	0.5 to >16	0.25 to >16	0.5 to >16
MIC_{50}		2	8	2	2	2
MIC_{90}		16	>16	4	>16	>16
Cotrimoxazole	S ≤ 2					
Susceptible [*n* (%)]		39 (97.5)	12 (100)	11 (100)	9 (100)	7 (87.5)
MIC range		<0.25 to >8	<0.25 to 2	<0.25 to 2	<0.25 to 2	<0.25 to >8
MIC_{50}		0.5	1	2	<0.25	1
MIC_{90}		2	2	1	2	>8
Doxycycline	S ≤ 1					
Susceptible [*n* (%)]		15 (37.5)	3 (25)	7 (58.3)	0	5 (62.5)
MIC range		<0.12 to >16	<0.12 to >16	<0.12 to 4	2 to >16	<0.12 to 8
MIC_{50}		2	4	1	4	1
MIC_{90}		8	8	4	>16	8
Imipenem	S ≤ 4					
Susceptible [*n* (%)]		27 (67.5)	6 (50)	8 (72.7)	9 (100)	4 (50)
MIC range		<1 to >64	<2 to 64	<2 to 32	<2 to 4	<1 to >64
MIC_{50}		<2	4	<2	<2	4
MIC_{90}		64	>64	32	4	>64
Linezolid	S ≤ 8					
Susceptible [*n* (%)]		40 (100)	12 (100)	11 (100)	9 (100)	8 (100)
MIC range		<1 to 8	<1 to 8	<1 to 4	<1 to 4	<1 to 4
MIC_{50}		<1	<1	<1	<1	<1
MIC_{90}		4	4	4	4	4
Minocycline	S ≤ 1					
Susceptible [*n* (%)]		18 (45)	4 (33.3)	7 (63.6)	1 (11.1)	6 (75)
MIC range		<1 to >8	<1 to >8	<1 to 4	1–4	<1 to 2
MIC_{50}		2	2	<1	2	<1
MIC_{90}		4	4	4	4	2
Moxifloxacin	S ≤ 1					
Susceptible [*n* (%)]		16 (40)	8 (66.7)	1 (9.1)	1 (11.1)	6 (75)
MIC range		<0.25 to >8	<0.25 to >8	<0.25 to 4	1 to >8	<0.25 to 8
MIC_{50}		2	0.5	2	4	0.5
MIC_{90}		4	4	4	>8	8
Tigecycline	S ≤ 0.5					
Susceptible [*n* (%)]		21 (52.5)	5 (45.5)	7 (70)	5 (55.5)	4 (50)
MIC range		0.12–4	0.12–4	0.25–1	0.5–2	0.25–2
MIC_{50}		0.5	1	0.5	0.5	0.5
MIC_{90}		2	4	1	2	2
Tobramycin	S ≤ 4					
Susceptible [*n* (%)]		21 (52.5)	1 (8.3)	11 (100)	2 (22.2)	7 (87.5)
MIC range		<1 to >16	<1 to >16	<1 to 2	<1 to >16	<1 to >16
MIC_{50}		2	8	<1	>16	<1
MIC_{90}		16	>16	1	>16	>16

Table 2
$MIC5_0$, MIC_{50}, and range (mg/L) and percentage of susceptible isolates of *Gordonia* spp. and *Tsukamurella* spp.

Antimicrobial agent	Breakpoints	Species (no. of strains tested)		
		G. sputii (*n* = 8)	*G. bronchialis* (*n* = 4)	*Tsukamurella* spp. (*n* = 3)
Amikacin	S ≤ 8			
Susceptible [*n* (%)]		5 (83.3)	4 (100)	3 (100)
MIC range		<1 to 32	<1	<1 to 2
MIC_{50}		<1	<1	<1
MIC_{90}		32	<1	2
Amoxicillin-clavulanate	S ≤ 8			
Susceptible [*n* (%)]		6 (100)	3 (75)	0
MIC range		<2 to 8	<2 to >64	64 to >64
MIC_{90}		<2	<2	>64
MIC_{50}		8	>64	>64
Cefoxitin	S ≤ 16			
Susceptible [*n* (%)]		3 (50)	1 (25)	0
MIC range		8– 128	<4 to 128	128
MIC_{50}		32	32	128
MIC_{90}		128	128	128
Ceftriaxone	S ≤ 8			
Susceptible [*n* (%)]		6 (100)	3 (75)	1 (33.3)
MIC range		<4 to 16	<4 to >64	8 to >64
MIC_{50}		<4	<4	16
MIC_{90}		16	>64	>64
Ciprofloxacin	S ≤ 1			
Susceptible [*n* (%)]		5 (83.3)	4 (100)	3 (100)
MIC range		<0.12 to 2	<0.12 to 0.25	<0.12 to 1
MIC_{50}		<0.12	<0.12	1
MIC_{90}		2	0.25	1
Clarithromycin	S ≤ 2			
Susceptible [*n* (%)]		6 (100)	2 (50)	2 (66.6)
MIC range		0.12–1	0.5 to >16	0.25–4
MIC_{50}		0.25	0.5	0.25
MIC_{90}		1	>16	4
Cotrimoxazole	S ≤ 2			
Susceptible [*n* (%)]		5 (83.3)	4 (100)	2 (66.6)
MIC range		<0.25 to >8	<0.25 to 1	<0.25 to >8
MIC_{50}		0.5	<0.25	<0.25
MIC_{90}		>8	1	>8
Doxycycline	S ≤ 1			
Susceptible [*n* (%)]		6 (100)	2 (50)	1 (33.3)
MIC range		0.25 to 1	<0.12 to 2	<0.12 to 4
MIC_{50}		0.5	1	4
MIC_{90}		1	2	4
Imipenem	S ≤ 4			
Susceptible [*n* (%)]		5 (83.3)	2 (50)	1 (33.3)
MIC range		<2 to 32	<2 to 32	<2 to 32
MIC_{50}		<2	<2	16
MIC_{90}		32	32	32
Linezolid	S ≤ 8			
Susceptible [*n* (%)]		6 (100)	4 (100)	3 (100)
MIC range		<1 to4	<1 to 2	<1 to 4
MIC_{50}		<1	<1	2
MIC_{90}		4	2	4
Minocycline	S ≤ 1			
Susceptible [*n* (%)]		5 (83.3)	1 (25)	2 (66.6)
MIC range		<1 to 4	<1 to 4	<1 to 2
MIC_{50}		<1	2	<1
MIC_{90}		4	4	2
Moxifloxacin	S ≤ 1			
Susceptible [*n* (%)]		5 (83.3)	4 (100)	3 (100)
MIC range		<0.25 to 8	<0.25 to 1	<0.25
MIC_{50}		<0.25	<0.25	<0.25
MIC_{90}		8	1	<0.25
Tigecycline	S ≤ 0.5			
Susceptible [*n* (%)]		3 (50)	2 (50)	3 (100)
MIC range		0.06–2	0.25–2	0.25–3
MIC_{50}		0.5	0.5	0.5
MIC_{90}		2	2	0.5
Tobramycin	S ≤ 4			
Susceptible [*n* (%)]		6 (100)	4 (100)	1 (33.3)
MIC range		<1 to 2	<1 to 2	<1 to 16
MIC_{50}		<1	<1	8
MIC_{90}		2	2	16

database coverage.[11,13] In fact, in several laboratories, modified protocols or in-house created libraries are used.[8,16] For instance, our Vitek MS IVD database cannot distinguish between *N. nova* and *N. africanum*, as they are closely related species from the *N. nova* complex.

During the study period, the most predominant *Nocardia* species were *N. farcinica* (30%), *N. ciryacigeorgica* (27.5%) and *N. nova/africanum* (22.5%). The predominance of these species has also been reported in previous Spanish studies,[9,17] even though there could be local differences in the epidemiology of the different species. Interestingly, in those studies, *N. abscessus* accounts for around 10–15% of nocardiosis cases, and we only had one case in our series.

Overall, amikacin and linezolid were the most active drugs, which is in accordance with previous reports.[9,18] Cotrimoxazole is usually the treatment of choice for *Nocardia* infections, although increasing resistance has been reported.[19] In the present study, the resistance rate still remains low (2.5%) in agreement with data reported for our country.[9]

Even though imipenem is considered as a first-line alternative for the treatment of nocardiosis, we observed that 32.5% of the isolates were resistant *in vitro*, including 50% of *N. farcinica* strains and 27.3% of *N. cyriacigeogica* strains, the most frequentlty isolated species in this study. Regarding other β-lactams, the susceptibility of ceftriaxone was variable, susceptibility to amoxicillin was low (30%), and almost all isolates (77.5%) were resistant to cefoxitin. Resistance to tetracyclines remains moderate in comparison with other data previously reported, being tigecycline the most active drug. We found a high percentage of quinolone-resistant isolates, being susceptibility rates slightly raised for moxifloxacin. Although all isolates were susceptible to amikacin, approximately only half of them (52.5%) were tobramycin susceptible.

Information in the literature regarding the identification by MALDI-TOF and antimicrobial susceptibility of aerobic actinomycetes other than *Nocardia* spp. is scarce. Optimal treatment of infections caused by *Gordonia* spp. and *Tsukamurella* spp. remains unclear, mainly because of the lack of experience due to the low number of cases of human disease caused by these genera. In our series we obtained nine isolates from sputum (*Gordonia* spp. $n=7$, *Tsukamurella* spp. $n=2$), three from blood (*Gordonia* spp. $n=2$, *Tsukamurella* spp. $n=1$), and one from a wound exudate (*Gordonia* spp.). Among our isolates, we found that aminoglycosides, fluoroquinolones, and linezolid showed good activity against *Gordonia* spp. The MIC values of the antibiotics tested were generally low except for cefoxitin. For *Tsukamurella* spp., in this study, the MIC values were variable between the three isolates, being amikacin, ciprofloxacin, moxifloxacin, and linezolid the most active drugs.

A major limitation of our study is the small number of isolates, especially those that are less-common species. This study was performed only in one center of the area and may not represent the epidemiology of the whole region, although these data will aid in future studies and it will be helpful for the understanding of infections caused by these microorganisms.

In conclusion, in our study, most of the *Nocardia* spp. isolates are susceptible to the recommended antibiotics for treatment (cotrimoxazole, amikacin, imipenem, linezolid). The accuracy in the identification of these species can be easily obtained with the MALDI-TOF technology. The identification together with the determination of its *in vitro* susceptibility could be critical to ensure a successful treatment and outcome. Moreover, these results will improve our knowledge of different aspects regarding these uncommon isolates in our area.

Ethical approval, consent to participate and consent to publish

The present study included only strains, and according to the current laws, it needs no approval from the ERC. No specific consents for participation and publication are also needed according the laws.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contribution

LS-V, MM-G, AM-V performed the susceptibility study and some of the MALDI-ToF identifications, AP performed all other MALDI-ToF identifications, LS-V supervised the MALDI-ToF identification and wrote the draft of the manuscript, JE has the idea, supervised all the project and reviewed the manuscript until its final version. All authors agreed with the content of the manuscript and to participate in its publication.

Competing interests

JE received travel grants from bioMérieux. No other competing interests related with the present manuscript need to be declared.

Acknowledgements

The technical assistance of A. Bodas and E. Ruiz is gratefully acknowledged. We want to acknowledge the Centro Nacional de Microbiología (Majadahonda, Spain), for its help with the identification of some isolates.

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