ORIGINAL ARTICLE

Identification and antimicrobial susceptibility of *Streptomyces* and other unusual *Actinobacteria* clinical isolates in Spain

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Abstract

Two hundred and eighty-six isolates from human clinical samples were identified between 1996 and 2019 as belonging to 8 families, 19 genera and 88 species of *Actinobacteria*. The most identified genera were *Streptomyces* (182 strains from 45 species), *Actinomadura* (29 strains, 5 species), *Nocardiopsis* (21 strains, 6 species) and *Dietzia* (18 strains, 5 species). The rest of the identified genera (15) contained 27 species with 36 isolates. Of the species studied, only 13/88 had been documented previously as isolates from clinical samples, and in some cases, as true pathogens. In this sense, a literature review of the species found in infections or in clinical samples without clear involvement in pathology has been carried out. Finally, the susceptibility to 8 antimicrobial agents has been studied. *Streptomyces* showed high resistance (80.8%) against cefotaxime and cotrimoxazole (55.5%), and no isolate resistance to amikacin and linezolid have been found. Lower percentages of resistance have been found in other genera, except in *Dietzia* (100% against cotrimoxazole and 44.4% against erythromycin). The greatest resistance in these genera was to cotrimoxazole (29.8) and erythromycin (27,9%), and no resistance to linezolid has been found in these genera. In *Microbispora, Nonomuraea and Umezawaea*, no resistant isolates have been found against any antibiotic studied. Only 3/104 isolates were resistant to amikacin in *Amycolatopsis*, *Crossiella*, and *Micromonosopora.* One isolate of *Amycolatopsis* was resistant to imipenem.

Keywords: *Actinobacteria*, Actimomadura, antimicrobial susceptibility, human clinical samples, *Streptomyces*
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Introduction

Aerobic Actinomycetes are habitual inhabitants of the soil and other environments. They are Gram-positive, generally filamentous and partially acid-alcohol resistant, being *Nocardia*, *Tsukamurella,* and *Gordonia* the most frequently associated with true infections in humans and animals [1]. However, species of other genera have been more frequently isolated and associated with clinical samples and many of them implicated in clinical cases of infection, especially in immunocompromised patients. In the case of *Streptomyces*, actinomycetomas and respiratory infections, and many others have been documented [1]. In this genus, although 671 species have currently been described (https://lpsn.dsmz/genus/streptomyces), only a few have been reported as clinical isolates with relevance in human infection. Something similar occurs to other genera such as *Actinomadura, Nocardiopsis* and *Dietzia* [1,2]. In the present study, we have identified isolates of these genera and others, isolated from human clinical samples received in our Taxonomy Reference Laboratory from hospital laboratories from Spain between 1996 and 2019. We have also carried out a review of the species and genera previously published in the literature.

Material and methods

Bacterial strains and clinical samples sources

Two hundred eighty-six *Actinobacteria* isolates from clinical samples from 61 hospital and public health laboratories from 33

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Spanish provinces were submitted to our Taxonomy Reference Laboratory for identification between 1996 and 2019. The 182 *Streptomyces* isolates were collected mostly from respiratory samples: being sputum and bronchoalveolar lavage/bronchial aspirate(BAL/BAS), most frequently isolated (117). The rest of the isolates were recovered from 17 sources; some of them were as relevant as blood, cerebrospinal fluid or brain abscess (Table 1). Five isolates were found from laboratory surface, catheter tips, laminar flow, and bone prosthesis.

In the distribution of the 104 isolates from other genera were identified as respiratory samples (67 isolates), and the rest (34) were distributed in 12 sources, highlighting blood with 11 isolates. Seven isolates were isolated from laboratory surface, air, and water (Table 2).

All received isolates were plated on Columbia Agar with 5% defibrinated sheep blood and incubated for 24–72 h in aerobic conditions at 37 °C. Some fastidious isolates of *Micromonospora, Actinomadura*, *Nonomuraea, Marinactinospora* and *Amycolatopsis* required a longer incubation period of 72 h to 7 days. All isolates except *Dietzia* strains (cocci-bacilli) were filamentous Gram-positive bacilli, partially acid-alcohol resistant with grey, white, or pigmented agar-adherent colonies.

Genes for molecular identification of species

Genes were used at three levels for the molecular identification of the species. The first gene used to identify all the isolates was the 16S rRNA gene. A 1300–1400 bp fragment was obtained according to the previously described methodology [3]. In the

TABLE 1. Sources of clinical samples of *Streptomyces sp.* identified in Spain (1996-2019) (45 species) (n = 182 isolates)

		Respiratory sources									
Species	**N°**	**Sputum**	**BAS/BAL[a]**	**Others[b]**	**Wounds**	**Blood**	**SF**	**Otic**	**Eye[c]**	**Others[d]**	**Environmental[e]**
S. albidoflavus	25	12	8		2		1		1	1	
S. albogriseolus	2	2			1						
S. albus	23	11	4	2	1	3		1			1
S. bacillaris	3	1						2			
S. cacaoi	22	13	3		2		1	3			
S. carpaticus	2	1				1					
S. cavourensis	2	1								1	
S. flaveolus	2		1		1						
S. fulvissimus	5	3	1								1
S. harbibensis	2	1	1								
S. olivaceus	4		1	1	1						1
S. puniceus	3	2					1				
S. pratensis	2	1		1							
S. rochei	23	15	**5**	2	1						
S. rutgergensis	5	4			1						
S. thermoviolaceus	19	5	4			4	3		1	1	
S. youssoufuensis	2		1			1					
S. albaduncus	1						1				
S. alboniger	1									1	
S. ambofaciens	1	1									
S. andamanensis	1			1							
S. araujoniae	1	1									
S. bobili	1									1	
S. celullosae	1	1									
S. cinereoruber	1	1									
S. drozdowiczii	1	1									
S. diastaticus	1	1									
S. endophyticus	1								1		
S. fragilis	1										1
S. globisporus	1		1								
S. griseoflavus	1			1							
S. griseoruber	1	1									
S. kanamyceticus	1				1						
S. longisporoflavus	1			1							
S. marokkonensis	1	1									
S. matensis	1	1									
S. misionensis	1		1								
S. netropsis	1				1						
S. nigra	1						1				
S. peucetius	1					1					
S. tauricus	1				1						
S. thermocarboxidus	1								1		
S.thermocoprophilus	1				1						
S. vastus	1									1	
S. xylaniliticus	1	1									
Streptomyces sp.	8	2	2						1	2	1
Total	182	84	33	9	14	10	8	6	5	8	5

Abbreviations: SF, spinal fluid.
[a]BAS/BAL: Bronchial aspirate/bronchoalveolar lavage, SF, spinal fluid.
[b]Lung(3), oral biopsy, nasal abscess, pleura(4).
[c]Conjunctival exudate (2), Granuloma, Vitreous humour, corneal scraping.
[d]Pericardial fluid(2), synovial fluid(2), bile fluid, brain abscess, liver abscess(2).
[e]Environmental samples: laboratory surface(2), catheter tips, laminar flow cabinet, bone prosthesis.

TABLE 2. Sources of clinical isolates of other *Actinobacteria* (18 genera, 45 species) (n = 104 isolates)

		Respiratory sources								
Species	**N°**	**Sputum**	**BAS/BAL[a]**	**Others[b]**	**Wounds**	**Blood**	**SF[c]**	**Eye[d]**	**Others[e]**	**Environmental.[f]**
Actinomadura:	**29**									
A. bangladeshensis	3	3								
A. cremea	6	5	2							
A. geliboluensis	8	6								
A. madurae	2	1	1		1					
A. meyerae	1				1					
A. nitritigenes	7	6			1					
A. rifamycini	1	1								
A. yumaniensis	1	1								
Nocardiopsis:	**21**									
N. alba	4	4								
N. dassonvillei	12	9	1			1		1		
N. prasina	1	1								
N. synemataformans	2	1	1							
N. tropica	1	1								
N. umidischolate	1		1							
Dietzia:	**18**									
D. aerolata	1								1	
D. cinnamea	10			1		3	1		1	4
D. maris	5								3	2
D. natronolimaea	1					1				
D. timonensis	1					1				
Saccharomonospora:	**8**									
S. azurea	4			1		2		1		
S. cyanea	1	1								
S. glauca	1				1					
S. viridis	2	2								
Saccharopolyspora:	**7**									
S. gloriosae	3	1			1	1				
S. hirsuta	1	1								
S. hordei	2	1							1	
S. pogona	1	1								
Microbispora:	**3**									
M. mesophila	1		1							
M. rosea	2	1	1							
Kitasatospora	**2**									
K. aburaviensis	1								1	
K. phosalacinea	1	1								
Marinactinospora:	**2**									
M. endophytica	1	1								
M. thermotolerans	1	1								
Pseudonocardia:	**2**									
P. carboxydivorans	1						1			
P. kongjuensis	1								1	
Amycolatopsis:	**2**									
A. cappadoca	1						1			
A. japónica	1								1	
Saccharotrix:	**2**									
S. longispora	1			1						
S. texasensis	1				1					
Aeromicrobium:										
A. massiliense	2								1	1
Nonomuraea:										
N. fastidiosa	1								1	
Crosiella:										
C. equi	1	1								
Prauserella:										
P. rugosa	1	1								
Sciscionella:										
S. marina	1					1				
Umezawaea:										
U. tangerina	1				1					
Micromonospora:										
M. echinospora	1					1				
Total	104	52	8	3	7	11	3	2	11	7

[a]BAS/BAL: Bronchial aspirate/bronchoalveolar lavage.
[b]Pleural fluid (3).
[c]Spinal fluid.
[d]Corneal ulcer(2).
[e]Pericardial fluid (2), synovial fluid (2), ascitic fluid, peritoneal fluid, Urine, bone marrow, brain abscess, ganglion, cervical ulcer.
[f]Laboratory surface (3), laboratory water, air (3).

isolates that showed a lack of differentiation between species with the 16S rRNA gene, a fragment of 800–900 bp of the *rpo*B gene was obtained and studied according to the conditions previously described [4]. Lastly, specific primers to 16S rRNA were used to improve the identification of the species of some genera, such as *Pseudonocardia*, *Saccharopolyspora*, and *Amycolatopsis* [5]

The fragment sequenced for each isolate were compared to sequences in the GenBank database and identified using BLAST (version 2.2.10 see http://www.ncbi.nlm.nih.gov/BLAST). A

similar score of ≥99.6% between the 16S *r*RNA and *rpo*B sequences and database sequences was deemed to indicate that isolate belonged to the same species [6].

Antimicrobial susceptibility

Susceptibility to eight antimicrobials (amoxicillin/clavulanic acid, cefotaxime, cotrimoxazole, amikacin, erythromycin, ciprofloxacin, linezolid and imipenem) was determined by E-test (Biomerieux, France) on Mueller-Hinton agar plates of 15 mm with 5% of defibrinated sheep blood, incubated in aerobiosis at 37 °C and read after 48 h. or more if it required in some fastidious isolates. The inoculum preparation in order to prevent the formation of irregular clumps was carried up as previously described [6]. The interpretative criteria as susceptible, intermediately resistant or resistant was made according to the breakpoints recommended by the CLSI for *Nocardia* and other Actinomycetes [7].

Results and discussion

The identification of isolates of *Streptomyces* and other *Actinobacteria* isolated from clinical samples from 61 hospital and public health laboratories from 33 provinces in Spain have been studied. Between 1996 and 2019, 286 isolates have been identified, belonging to 19 genera and 88 species, encompassed in 9 families. All isolates were gram-positive filamentous microorganisms, except for isolates of the genus *Dietzia*. The most common genera found were *Streptomyces* (182 isolates and 45 species), *Actinomadura* (29 and 5), *Nocardiopsis* (21 and 6) and *Dietzia* (18 and 5). The rest of the genera found were 36 isolates of 15 genera, including 27 different species (Table 3).

The identification of *Streptomyces* and other aerobic actinomycetes, as indicated in the CLSI guidelines of criteria for the identification of bacteria by DNA target sequencing [8], presents limitations for several reasons: information on sequences in the databases, the high similarity between sequences of different species. This fact made the diagnostic information only recommended with their genus is usually sufficient [8]. However, in recent years, the databases of both individual genes and complete genomes belonging to *Actinobacteria* have increased significantly. In addition, there has also been a notable increase in publications that mention isolation findings in human clinical samples and cases of infection caused by *Streptomyces* and other similar genera.

Extensive taxonomy studies have also been carried out using other alternative or complementary genes to the 16S rRNA gene, such as *gyr*B and *rpo*B, which have allowed the identification of some *Nocardia* species and extensive phylogeny studies [9,10]. In addition, multilocus sequence analysis has allowed us to more precisely know the structure and relationship of *Streptomyces* and other genera [11,12].

Streptomyces

In our study, we have identified 182 *Streptomyces* isolates isolated from clinical samples that are shown in Table 1. Most of the samples were respiratory (sputum, BAL/BAS, pleura, lung and others). We have identified 45 species; 28 of them (2%) were represented by one isolate. The most frequently identified were *S. albidoflavus* (25 isolates), *S. albus* (23), *S. cacaoi* (22), *S. rochei,* (23), and *S. thermoviolaceus* (19). *S. albidoflavus*, *S. rochei,* and *S. thermoviolaceus* had not been previously cited as isolated from clinical samples. Of the rest of the identified species (Table 1), only *S. olivaceus* and *S. thermocarboxidus* have

TABLE 3. Unusual *Actinobacteria* isolates from human clinical samples in Spain (1996-2019)

Family	Genera	Isolates	N° sp[a]	Bergey's[b]	LPSN[c]	Clinical isolates[d]
Streptomycetaceae	*Streptomyces*	182	45	533	672	**Yes**
Streptomycetaceae	*Kitasatospora*	2	2	20	32	
Nocardiopsaceae	*Nocardiopsis*	21	6	30	44	**Yes**
Nocardiopsaceae	*Marinactinospora*	2	2	—	2	
Thermomonosporaceae	*Actinomadura*	29	5	39	67	**Yes**
Streptosporangiae	*Nonomuraea*	1	1	24	60	
Streptosporangiae	*Microbispora*	3	2	13	10	
Micrommonosporaceae	*Micromonospora*	1	1	32	105	**Yes**
Nocardiopdaceae	*Aeromicrobium*	2	1	10	19	**Yes**
Pseudonocardiaceae	*Pseudonocardia*	2	2	29	60	**Yes**
Pseudonocardiaceae	*Amicolatopsis*	2	2	40	80	**Yes**
Pseudonocardiaceae	*Crosiella*	1	1	2	2	
Pseudonocardiaceae	*Prauserella*	1	1	9	5	
Pseudonocardiaceae	*Saccharomonospora*	8	4	9	14	**Yes**
Pseudonocardiaceae	*Saccharopolyspora*	7	4	20	33	
Pseudonocardiaceae	*Saccharotrix*	2	2	9	21	
Pseudonocardiaceae	*Sciscionella*	1	1	—	1	
Pseudonocardiaceae	*Umezawaea*	1	1	1	2	
Dietziae	*Dietzia*	18	5	11	11	**Yes**
Total		286	88	831	1240	

[a]Species identified from the National Reference of Taxonomy Laboratory from clinical samples.
[b]Species described in the last edition os Bergey's Manual (2012).
[c]Species registered in LPSN-dsmz.de (2020). (https://lpsn.dsmz/genus/streptomyces).
[d]genera with species isolated from human clinical samples reported in the literature.

been previously identified in clinical samples [15,16]. Most of the isolates of the 45 *Streptomyces* species identified had one or few isolates. The most identified species were *S. albidoflavus, S. albus, S. rochei, S. cacaoi,* and *S. thermoviolaceus.* From the point of view of their source of the isolates, sputum and other respiratory samples are clearly the majority (69%), followed by wounds and abscesses and blood. The rest of the isolates belonged to nine other different sources. These included some as relevant as spinal fluid, brain abscess, pericardial fluid, or liver abscess (Table 1).

Resistance (intermediately resistant and resistant) against eight antimicrobials out of 182 *Streptomyces* isolates and 104 isolates from other genera are shown in Tables 5 and 6. In the 45 studied species (182 isolates) of *Streptomyces,* no resistant isolates against amikacin and linezolid have been found. Only 5.5% of the isolates were resistant to imipenem. Between 11.0% and 35.0% of the isolates were resistant to ciprofloxacin, erythromycin, and amoxicillin/clavulanic acid. The high resistance figures were found to be cotrimoxazole (55.5%) and cefotaxime (80.8%). In many species studied, resistance to cefotaxime was 100%, as well as in species with an appreciable number of isolates (*S. cacaoi*, *S. rochei)* and others with a smaller number of isolates as *S. fulvissimus, S. olivaceus, S. rutgergensis*. The 19 isolates of *S. thermoviolaceus* only showed resistance against cefotaxime (15.8%) and cotrimoxazole (68.4%).

The 16S rRNA gene has been very useful for the identification of genera and species in most cases. Although in some of them the *rpo*B gene has been required as a complementary analysis, *S. rochei* isolates cannot be differentiated from other species such as *S. enissocaesilis, S. plicatus, S. vinaceodrappus* and *S. mutabilis*) by the gene sequence 16s rRNA, because these species are phylogenetically very close and included in Clade 119 by Labeda et al. [11]. Later phylogenetic studies established that these species were later synonyms of *S. rochei* [12]. In any case, the use of the *rpo*B gene sequences allowed us to discriminate and definitely identify this species. Something similar happened with the differentiation between isolates of *S. cacaoi* and *S. violaceoruber.*

The extensive genus of *Streptomyces* includes microorganisms mainly from the soil and other environments. Its interest

TABLE 4. Genera and species isolated from human clinical samples in Spain (1996-2019)

Genera/Species	N° clinical strains	Source[a]	Source (type strain)	Clinical samples[b]	References
Streptomyces:	**182**				
S. albidoflavus	25	Sputum	ND[c]		
S. albus	23	Sputum	Soil	Yes	[17,22,25]
S. albogriseolus	2	Sputum	Soil		
S. bacillaris	3	Otic exudate	Soil		
S. cacaoi	22	Sputum	Cocoa	Yes	[29,30]
S. carpaticus	2	Sputum/blood	ND		
S. cavourensis	2	Sputum/bile	Soil		
S. flaveolus	2	BAS/wound	ND		
S. fulvissimus	5	Sputum	ND		
S. harbibensis	2	Sputum	Soybean root		
S. olivaceus	4	BAS/Lung	Soil		
S. puniceus	3	Sputum	ND		
S. pratensis	2	Sputum/lung	Compost		
S. rochei	23	Sputum	Soil		
S. rutgergensis	5	Sputum	Soil		
S. youssoufuensis	2	Sputum	Mine soil		
S. thermoviolaceus	19	Sputum/blood	Soil		
S. albaduncus	1	Spinal fluid	ND		
S. alboniger	1	Pericardial fluid	Soil forest		
S. ambofaciens	1	Sputum	Soil		
S. andamanensis	1	Lung	Soil		
S. araujoniae	1	Sputum	Potato		
S. bobili	1	Brain abscess	Soil		
S. celullosae	1	Sputum	Garden soil		
S. cinereoruber	1	Sputum	Soil		
S. drozdowiczii	1	Sputum	Forest soil		
S. diastaticus	1	Sputum	Soil		
S. endophyticus	1	Cornea	Plants		
S. globisporus	1	BAS	Soil		
S. fragilis	1	Surface lab	Soil		
S. griseoflavus	1	Nasal exudate	Soil		
S. griseorubens	1	Sputum	Soil		
S. kanamyceticus	1	Ulcer	ND		
S. longisporoflavus	1	Pleural fluid	Soil		
S. marokkonensis	1	Sputum	Rhizosphere		
S. matensis	1	Sputum	Sea sand		
S. misionensis	1	BAS	Soil		
S. netropsis	1	Wound	ND		
S. nigra	1	Spinal fluid	Soil		
S. peucetius	1	Blood	Soil		
S. tauricus	1	Wound	ND		
S. thermocarboxidus	1	Conjunctiva	Soil	Yes	[33]
S.thermocoprophilus	1	Wound	Bird faeces		
S. vastus	1	Pericaldial fluid	Waste		
S. xylaniliticus	1	Sputum	Soil		

Continued

TABLE 4. Continued

Genera/Species	Nº clinical strains	Source[a]	Source (type strain)	Clinical samples[b]	References
Streptomyces spp.	8	Sputum/liver abscess	Soil		
Actinomadura:	**29**				
A. bangladeshensis	3	Sputum	Soil		
A. cremea	6	Sputum	Soil	**Yes**	[43]
A. geliboluensis	8	Sputum	Soil		
A. madurae	2	Sputum/wound	Soil, Mycetoma	**Yes**	[39,41,42]
A. meyerae	1	Wound	Garden soil		
A. nitritigenes	7	Sputum	Biofilters	**Yes**	[43]
A. rifamycini	1	Sputum	Soil		
A. yumaniensis	1	Sputum	Soil		
Nocardiopsis:	**21**				
N. alba	4	Sputum	Hip drainage		
N. dassonvillei	12	Sputum	Conjunctiva	**Yes**	[47–56]
N. prasina	1	Sputum	Soil		
N. synemataformans	2	Sputum/BAL	Sputum	**Yes**	[57]
N. tropica	1	Sputum	Rhizosphere		
N. umidischolate	1	BAL	Indoor dust		
Dietzia:	**18**				
D. aerolata	1	Pericardial fluid	Duck air		
D. cinnamea	10	Blood/Environ.	Perianal swab	**Yes**	[63–65]
D. maris	5	Ascitic fluid/Bone marrow	Soil, skin, carp	**Yes**	[59–63]
D. natronolimaea	1	Blood	Sediments	**Yes**	[58]
D. timonensis	1	Blood	Soil		
Saccharomonospora:	**8**				
S. azurea	4	Blood	Soil		
S. cyanea	1	Sputum	Soil		
S. glauca	1	Wound	Compost, manure		
S. viridis	2	Sputum	Soil		
Saccharopolyspora:	**7**				
S. gloriosae	3	Blood/Sputum	Plant		
S. hirsuta	1	Sputum	Moldy sugarcane		
S. hordei	2	Sputum	Cereals		
S. pogona	1	Sputum	Soil		
Microbispora:					
M. mesophila	1	BAS	Soil		
M. rosea	2	Sputum/BAS	Soil		
Kitasatospora					
K. aburaviensis	1	Pericardial fluid	Soil		
K. phosalacinea	1	Sputum	Soil		
Marinactinospora:					
M. endophytica	1	Sputum	Plant		
M. thermotolerans	1	Sputum	Sea sediment		
Pseudonocardia:					
P. carboxydivorans	1	Spinal fluid	Soil	**Yes**	[76]
P. kongjuensis	1	Brain abscess	Gold mine		
Amycolatopsis:					
A. cappadoca	1	Spinal fluid	Soil		
A. japónica	1	Peritoneal fluid	Soil		
Saccharotrix:					
S. longispora	1	Pleural fluid	Soil		
S. texasensis	1	Wound	Soil		
Aeromicrobium:					
A. massiliense	2	Cervical ulcer/environ.	Human faeces	**Yes**	[81]
Nonomuraea:					
N. fastidiosa	1	Ganglion abscess	Hot spring silt		
Crossiella:					
C. equi	1	Sputum	Equine placenta		
Prauserella:					
P. rugosa	1	Sputum	Rumen cow		
Sciscionella:					
S. marina	1	Blood	Sea sediment		
Umezawaea:					
U. tangerina	1	Wound	Soil		
Micromonospora:					
M. echinospora	1	Blood	Soil		

[a]Representative sources in our study.
[b]Species registered from clinical samples in the literature.
[c]ND, no data.

lies in the fact that it is very important in biotechnological induction due to the ability to produce secondary metabolites of interest and mainly antibiotics. So far, there are 672 species described, and only a few have been isolated from clinical samples in humans, animals, and plants. Isolates from human clinical samples have been reported as *Streptomyces spp* due to the difficulties of discrimination between numerous species, whereas in the oldest reports in which the species appeared, identification was carried out by phenotypic methods [13,14]. Later and due to the development of molecular identification methods, until obtaining whole genomes, the identification of species is frequently increasing, especially in samples of human origin. Although in most cases the isolates from clinical samples are considered saprophytes, the implications of *Streptomyces* in

TABLE 5. Resistance of 182 *Streptomyces sp.* isolates from human clinical samples isolated in Spain (1996-2019)

Specie	Isolates	Amc	%R	Cfx	%R	Imp	%R	Amk	%R	Ery	%R	Cip	%R	Lnz	%R	SxT	%R
S. albidoflavus	25	7	**28.0**	19	**76.0**	2	**8.0**	—	—	6	**24.0**	1	**4.0**	—	—	16	**64.0**
S. albogriseolus	2	2	**100**	2	**100**	—	—	—	—	2	**100**	—	—	—	—	1	**50**
S. albus	23	8	**34.8**	20	**86.9**	—	—	—	—	5	**21.7**	1	**4.3**	—	—	1	**4.3**
S. bacillaris	3	—	—	3	**100**	—	—	—	—	3	**100**	—	—	—	—	3	**100**
S. cacaoi	22	2	9,1	22	**100**	—	—	—	—	12	**54.5**	4	**18.2**	—	—	17	**77.3**
S. carpaticus	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	**50**
S. cavourensis	2	1	**50**	2	**100**	—	—	—	—	—	—	—	—	—	—	1	**50**
S. flaveolus	2	1	**50**	1	**50**	—	—	—	—	1	**50**	—	—	—	—	1	**50**
S. fulvissimus	5	4	**80**	5	**100**	—	—	—	—	1	**20**	2	**40**	—	—	3	**60**
S. harbibensis	2	—	—	1	**50**	—	—	—	—	—	—	—	—	—	—	2	**100**
S. olivaceus	4	3	**75**	4	**100**	—	—			1	**25.0**	—	—	—	—	1	**25**
S. puniceus	3	2	**66.7**	3	**100**	—	—	—	—	2	**66.7**	—	—	—	—	2	**66.7**
S. pratensis	2	1	**50**	2	**100**	—	—	—	—	1	**50.0**	—	—	—	—	2	**100**
S. rochei	23	16	**69.6**	23	**100**	6	**26.1**			6	**26.1**	7	**30.4**			10	**43.5**
S. rutgergensis	5	5	**100**	5	**100**	—	—	—	—	3	**60.0**	—	—	—	—	1	**20,0**
S. youssufiensis	2	1	**50**	2	**100**	—	—	—	—	2	**100**	1	**50**	—	—	2	**100**
S. thermoviolaceus	19	—	—	3	**15.8**	—	—	—	—	—	—	—	—	—	—	13	**68.4**
Other species[a]	28	6	**21.4**	22	**78.6**	—	—	—	—	4	**14.3**	4	**14.3**	—	—	18	**64.3**
Streptomyces spp.	8	5	**62.5**	8	**100**	2	**25**	—	—	3	**3.5**	—	—	—	—	6	**75**
Total	182	**64**	**35.2**	**147**	**80,8**	**10**	**5.5**	—	—	**52**	**28.6**	**20**	**11.0**	—	—	**101**	**55.5**

Abbreviations: Amc, amoxicillin/clavulanic acid, Ctx, cefotaxime, Imp, imipenem, Amk, amikacin, Ery, erythromycin, Cip, ciprofloxacin, Lnz, linezolid,.SxT, trimetoprim/sulfametoxazol (cotrimoxazole).
[a]See Table 4.

TABLE 6. Resistance of 104 unusual *Actinobacteria* (18 genera) isolates from human clinical samples recovered in Spain

Genus	Isolates	Amc	%R	Cfx	%R	Imp	%R	Amk	%R	Ery	%R	Cip	%R	Lnz	%R	SxT	%R
Actinomadura	29	12	**41.4**	9	**31.0**	—	—	—	—	8	**27.6**	—	—	—	—	1	**3.4**
Nocardiopsis	21	2	**9.5**	1	**4.8**	—	—	—	—	4	**19.0**	2	**9.5**	—	—	3	**14.3**
Dietzia	18	—	—	—	—	—	—	—	—	8	**44.4**	—	—	—	—	18	**100**
Saccharomonospora	8	1	**12.5**	3	**37.5**	—	—	—	—	1	**12.5**	—	—	—	—	—	—
Sacchraopolyspora	7	—		1	**14.3**	—	—	—	—	1	**14.3**	1	**14.3**	—	—	—	—
Other genera:	21	2	**9.5**	5	**23.8**	1	**4.8**	3	**14.3**	7	**33.3**	2	**9.5**	—	-	9	**42.8**
Microbispora	3	—		—		—		—		—		—		—		—	
Kitasatospora	2			1		—		—	—	—		—		—		1	
Marinactinospora	2	1		—		—		—		1		—		—		—	
Pseudonocardia	2	—		—		—		—		—		—		—		2	
Amycolatopsis	2	1		2		1		1		2						2	
Saccharotrix	2	—		—		—		—		1		—		—		1	
Aeromicrobium	2	—		—		—		—		—		—		—		2	
Nonomuraea	1	—		—		—		—		—		—		—		—	
Crosiella	1	—		—		—		1		1		1		—		—	
Prauserella	1	—		1		—		—		1		—		—		1	
Scscionella	1	—		1		—		—		1		1		—		—	
Umezawaea	1	—		—		—		—		—		—		—		—	
Micromonospora	1	—		—		—		1		—		—		—		—	
Total *Actinobacteria*	104	17	**16.3**	19	**18.3**	**1**	**1.0**	3	**2.9**	29	**27.9**	5	**4.8**	—	—	31	**29.8**
Total *Streptomyces*	182	64	**35.2**	147	**80.8**	**10**	**5.5**	—	—	52	**28.6**	20	**11.0**	—	—	101	**55.5**
Total	286	81	**28.3**	166	**58.0**	**11**	**3.8**	3	**1.0**	81	**28.3**	25	**8.7**	—	—	132	**46.1**

Abbreviations: Amc, amoxicillin/clavulanic acid, Ctx, cefotaxime, Imp, imipenem, Amk, amikacin, Ery, erythromycin, Cip, ciprofloxacin, Lnz, linezolid, SxT, trimetoprim/sulfametoxazole (cotrimoxazole).

cases of actinomycetoma, pneumonia, bacteremia, brain abscess, arthritis, pericarditis, peritonitis, lymphadenitis and others, with isolates from blood, skin, bronchoalveolar lavage, sputum, brain abscess and others have been published [14]. The species that have been isolated and identified in these processes are *S. albus, S. griseus, S. somaliensis, S. lanatus, S. atratus, S. viridis, S. coelicolor, and S. pelletieri* [15–24]. In many cases, they were reported as *Streptomyces spp.* [25–28]. Other infections in which other species are implicated are *S. cacaoi* (scalp abscess, otitis media [29,30], *S. thermovulgaris* bacteremia in Crohn's disease patient. [31], *S. bikiniensis* in bacteremia [32], and *S. thermocarboxidicus* in keratitis [33].

These findings show the great variety of species that can be found in clinical samples and that, in many cases, can be implicated in cases of infection. In this sense, it would be interesting to analyze the possible virulence factors that these species may contain, which may contribute to their involvement in cases in immunocompromised or immunocompetent patients. An interesting study has been carried out to detect and compare superoxide dismutases in clinical and soil isolates of *Streptomyces* as possible indications of virulence, as happens in other gram positives such as *Listeria*, *Nocardia asteroides,* and *Mycobacterium tuberculosis* [25].

There is very little published information on susceptibility to antibiotics in *Streptomyces* [14,34]. In a study with 28 isolates of *S. griseus* referred to the Atlanta CDC between 1985 and 1988, 43% of isolates resistant to amoxicillin/clavulanic acid, 57% to ciprofloxacin and 53% to cefotaxime, 19% to imipenem and 14% to erythromycin [34]. A subsequent study with 86 isolates also referred to the CDC between 2000 and 2004 and identified at genus level it was found that 49% of isolates were resistant to amoxicillin/clavulanic acid, 65% to cefotaxime, 54% to ciprofloxacin, 75% to erythromycin, 33% to imipenem, and 65% to cotrimoxazole [14].

In our susceptibility study of 182 isolates of 45 species of *Streptomyces*, no resistant isolates have been found against linezolid and amikacin, while the previous study is consistent [34]. Similar resistance figures were found for cotrimoxazole and amoxicillin/clavulanic acid and especially for cefotaxime (80.8%). Resistance figures for erythromycin, ciprofloxacin, and imipenem were significantly lower. These differences could be due, in some cases, to the diversity of species studied since we have not found isolates resistant to amoxicillin/clavulanic acid in *S. thermoviolaceus*, *S. bacillaris, S. carpaticus,* and *S. harbibensis* (28 isolates). We have also not found strains resistant to erythromycin in *S. carpaticus, S. cavourensis, S. harbibensis, and S. thermoviolaceus*. Only 7/45 species (20/182 isolates) were resistant to ciprofloxacin, and lower percentages were seen. Only 10 imipenem resistant isolates of 2 species were found (*S. albidoflavus, S. rochei)* and two isolates of *Streptomyces spp*.

Actinomadura

In the 29 isolates of *Actinomadura* studied, 8 species have been identified, with *A. geliboluensis* (8 isolates), *A. nitritigenes* (7), and *A. cremea* (6) being the most found. It is noteworthy that they were isolated from sputum 23/29 and bronchial aspirates (3/29). Only three isolates were collected from wounds (*A. madurae, A. meyerae,* and *A. nitritigenes* (Table 2).

The resistance figures were higher: 41.4% to amoxicillin/clavulanic acid, 31.0% to cefotaxime, and 27% to erythromycin. Resistance to cotrimoxazole was 3.4%. We did not find isolates resistant to amikacin, ciprofloxacin, linezolid, or imipenem.

This genus belonging to the *Thermosporaceae* family, comprises 67 species. The first reservoir is the soil and other environments [35]. However, some species are responsible for actinomycetomas consisting of suppurating tumefactions of the skin and subcutaneous tissues as a result of penetrating wounds with soil contamination. These actinomycetomas occur mainly in tropical and subtropical areas of Africa, Asia and South America such as India, Bangladesh, Mali, Senegal, Venezuela and Mexico and many others; although they have also been detected in many other countries in Europe and the United States with less incidence [36–38]. Three species of *Actinomadura* have been fundamentally implicated in the production of mycetomas (*A. madurae, A. latina, and A pelletieri*) [39,40]. In addition, these and some other species have also been implicated in other pathologies such as A. *madurae* with an invasive lung infection in a patient with AIDS [41]and peritonitis [42]. They have also been isolated from sputum and BAL of pulmonary infections (*A. cremea, A., nitritigenes, A. chibensis* [43], and *A. sputi* [44]). Of the seven species identified five had not been reported in the literature (*A. bangladeshensis, A. geliboluensis, A. meyerae, A. yumaniensis,* and *A. rifamyicini)*.

From the little information that can be found on susceptibility to antibiotics in *Actinomadura*, we can highlight the study of 42 isolates of *A. madurae* referred to the Atlanta CDC from isolates between 1985 and 1988 [34]. The obtained resistance figures were lower than 10% isolates against erythromycin, ciprofloxacin, cotrimoxazole and cefotaxime and somewhat higher amoxicillin/clavulanic acid and cotrimoxazole (19% and 13%). They did not find strains resistant to imipenem.

The differences found in the resistance figures could be due to the fact that in our series, only two isolates of *A. madurae* were studied. The rest, especially *A. cremea* (6 isolates) and *A. geliboluensis* (8 isolates) were more resistant to antibiotics studied.

Nocardiopsis

We have identified 21 isolates of 6 species of *Nocardiopsis,*12/21 were *N. dassonvillei* (57%), and 2/21 were identified as N. *synnemataformans*. The other four species (*N. alba, N. prasina, N.*

tropica, and *N.umidischolate*) were identified for the first time from human clinical samples. All isolates were recovered from respiratory samples, except for two of *N. dassonvillei* that were collected from blood and the eyes. In both species: *N. dassonvillei* and *N. synnemataformans*, the sources of the clinical samples are the same previously described in the literature [46–57].

We have not found information on susceptibility to antibiotics in isolates of *Nocardiopsis*. Our results indicate low resistance figures (<20%) (Table 6). We have not found isolates resistant to amikacin, linezolid, and imipenem. Only six isolates showed some resistance profile to antimicrobials (*N. alba* 4/4 isolates and *N. prasina and N. tropica)*. It can be noted that all the isolates identified of *N. alba* presented some resistance, one of them being resistant to four antibiotics (amoxicillin/clavulanic acid, cefotaxime, cotrimoxazole, and ciprofloxacin, one isolate was resistant to cotromoxazole, one to amoxicillin/clavulanic acid, and other to erythromycin. We have not found isolates resistant to any antibiotic tested in *N. dassonvillei*.

This genus belonging to the family *Nocardiopsaceae,* is made up of 44 species whose main habitat is saline and alkaline soils, although some species were isolated for the first time from human clinical samples. They can also cause infections in dogs and other animals [45].

As early as 1911, the first isolation was made in a patient with conjunctivitis [46]. Only *N. dassonvillei* and *N. synnemataformans* have been recovered from human samples. *N. dassonvillei* has been isolated from bacteremia [47], mycetomas and other skin infections [48–51], and respiratory infections [52–56]. In 1997, *N. synnemataformans* was isolated from the sputum of a kidney transplant patient [57].

Dietzia

In our study, 18 isolates of 5 species of *Dietzia* have been identified, those previously identified in clinical samples (*D. maris, D. cinnamea, D, natronolimnaea)* and another not previously isolated in clinical samples: D. *aerolata* (pericardium), *D. timonensis*). The most common species was *D. cinnamea* (10/18), followed by *D. maris* (5/18), Isolated from samples previously described in the literature (blood, spinal fluid, and others). Six isolates from the hospital laboratory environment and equipment were also identified (Table 2). From the susceptibility to antibiotics studied, we can highlight the 100% resistance to cotrimoxazole and 44.4% to erythromycin. In a previous study with 26 clinical and type isolates, 10 were resistant to cotrimoxazole [58].

The genus *Dietzia* constitutes the only genus of the family *Dietziae*. Nonacid-alcohol resistant coccoid bacteria with very similar characteristics to *Rhodococcus* from which they derive and whose species were recently described [2]. This genus consists of 11 species whose habitats are aquatic and terrestrial. Likewise, some species such as *D. maris* have been isolated as potential pathogens or endosymbionts of insects and dinoflagellates (2). Three species have been recognized as potential human pathogens: *D. maris, D. cinnamea,* and *D. papillomastosis.* Although other species have also been recovered from clinical samples as *D. chimae* or *D. natronolimnaea* (in blood, wounds, lung, vagina, thoracic fluid, urine, and others) [58]. *D. maris* was isolated from the ground and in the intestine of carp and other marine environments. In 1999, it was reported as a producer of infection in humans when it was isolated from the blood of an immunocompromised patient with septic shock [59]. It has also been indicated as a producer of prosthetic hip infection [60].

Other locations of *D. maris* infections have been: aortitis [61], bone marrow infection of an immunocompetent patient [62]. *D. cinnamea* was isolated from a perianal swab from a bone marrow transplant patient [63], pleural fluid infection [64], and from a dog bite wound [65]. *D. papillomatosis* was isolated from reticulated papillomatosis [66,67] and bacteremia [68]. Other cases in which the species has not been identified were: endophthalmitis [69], and pacemaker pocket infection [70]. Recently another species has been added to those found in clinical samples: *D. aurantiaca* was collected from cerebrospinal fluid from a 24-year-old woman in Sweden [71]. *D. natronolimaea* has also been reported as isolated from blood [58].

Other genera

Other genera and species identified (15 and 27) are listed in Table 4. *Pseudonocardiaceae* stands out with 9 genera found with 18 species, being the most abundant *Saccharomonospora* (8 isolates) and *Saccharopolyspora* (7 isolates). All these identified genera and species have not been previously described with clinical sources in the literature, except for some specific reports of *Saccharomonospora*, *Pseudonocardia,* and *Amicolatopsis* (Table 3).

Only 15 isolates came from respiratory samples, the majority from sputum. It is noteworthy that no respiratory isolates have been found in the 9 genera of the *Pseudonocardiacea*e family, except for 2 isolates of pleural fluid identified as *Saccharotrix longispora* and *Saccharomonospora azurea*. The rest of the isolates came from blood, spinal fluid, brain abscess, peritoneal fluid, pleural fluid, corneal ulcer, and wound (Table 2).

Only a few species have been isolated in clinical samples or implicated in some syndromes. Regarding to *Amycolatopsis*, *A. orientalis* (formerly *Nocardia orientalis)* has been isolated from spinal fluid [72] and *A. palatopharyngis* has also been isolated from the oropharynx [73]. *A. benzoatilytica* (formerly *A. orientalis*) has been isolated from submandibular mycetoma [74].

Species of the genus *Pseudonocardia* have also been isolated from clinical samples and implicated in pathology [75]. *P. carboxydivorans* has been isolated from cerebrospinal fluid [76]. It

has also been associated, along with species of other genera (*Saccharomonospora* and *Saccharopolyspora*), to hypersensitivity pneumonitis [77–80]. *Micromonospora* has also been cited as rarely isolated from clinical samples as early as 1990, in 6/266 isolates of aerobic actinomycetes submitted to the Atlanta CDC [34].

At last, *Aeromicrobium massiliense* has been isolated from the faeces of an asymptomatic patient [81]. Other genera and species identified in our study, *Microbispora, Kitasatospora, Marinactinospora, Saccharotrix*, *Nonomuraea, Crossiella, Prauserella, Sciscionella*, and *Umezawaea*, have not been previously mentioned in isolates from clinical samples.

From the point of view of antimicrobial susceptibility in these genera, it is difficult to compare with the data obtained in our study due to a limited number of isolates by species. Overall, we found 42.5% resistance to cotrimoxazole and 33% to erythromycin as the most outstanding figures. In a study with 38 strains of *Amicolatopsis spp*. and 22 of *Crossiella equi* collected from cases of placentitis in horses, we can compare with the results obtained with one strain of *C. equi* isolated from sputum and two strains of *Amycolatopsis* isolated from spinal fluid (*A. cappadoca*) and peritoneal fluid (*A. japonica)*. Our *C. equi* strain was resistant to amikacin, erythromycin, and ciprofloxacin, and in the study cited with isolates of animal origin, they found 100% of the isolates resistant to amikacin, 86.4% resistant to macrolides and 95.5% of resistance to ciprofloxacin. In the case of *Amycolatopsis*: *A. japonica* was resistant to amoxicillin/clavulanic acid, cefotaxime, cotrimoxazole, erythromycin, and imipenem, while *A. cappadoca* was resistant to cefotaxime, cotrimoxazole, amikacin, and erythromycin. In the 38 isolates of *Amicolatopsis* in the study mentioned, no resistance to cotrimoxazole was found, while resistance to the other antibiotics mentioned in different proportions was found [82]. At last, no resistance was found in *Microbispora* (3 strains), *Nonomuraea* (1), and *Umezawaea* (1) (Table 6).

Concluding remarks

Especially since the 90s of the last century, the isolation in clinical samples and the pathogenic potential of genus and species of actinomycetes in humans and animals have been increasing. The general causes are well known, such as the increase in immunodeficiency acquired by certain pathologies or treatments. Likewise, both new diagnostic technologies and therapies have also contributed to the increase in the report of these isolates. All these facts, together with the great advances that have taken place in the field of bacterial identification using molecular techniques, have led to the detection and precise characterisation of these emerging microorganisms from clinical samples and their possible involvement in disease.

The fact of 'exotic' genera from habitats far removed from humans and animals, not previously isolated in clinical samples would indicate that either they were not previously detected, or that these microorganisms were already present in other habitats, either for the reasons indicated above, they have increased their presence.

Therefore, it is necessary that the isolates of these genera and species be taken into account and studied in relation to their possible virulence factors that favour their invasiveness and subsequent development of infections. Likewise, it is important that the study of antimicrobial susceptibility of these isolates be generalized to determine the more effective treatment of infection, as well as, after the appearance of resistance, study their mechanisms and compare them with those of common microorganisms in pathology and its possible cross-transmission of antibiotic resistance.

Credit author statements

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Transparency declaration

None declared.

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