Antibiotic susceptibility testing and species identification of Nocardia isolates: a retrospective analysis of data from a French expert laboratory, 2010-2015

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Original article

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Key-words: *Nocardia*, disk diffusion, 16S, *hsp65*

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This work was presented in part at the 28th European Congress of Clinical Microbiology and Infectious Diseases, Madrid, Spain, 21 to 24 April 2018

ABSTRACT

Objectives. *Nocardia*, a Gram-positive bacterium, is responsible for rare and severe infections. Accurate microbiological data are essential to guide antibiotic treatment. Our primary objective was to describe species identification and results of antimicrobial susceptibility testing (AST) for *Nocardia* isolates analysed over a 6-year period. Secondary objectives were to study temporal trends in species distribution and AST results.

Methods. We retrospectively analysed results from *Nocardia* isolates sent between January 2010 and December 2015 to a French laboratory dedicated to *Nocardia* (Observatoire Français des Nocardioses). Species identification was obtained by amplification and sequencing of a 600bp fragment of the 16S rRNA gene (for all isolates) and of *hsp65* (when required). AST was performed using disk diffusion.

Results. We included 793 *Nocardia* isolates, mostly from the lungs (53.8%). The most frequent species were *N. farcinica* (20.2%), *N. abscessus* complex (19.9%) and *N. nova* complex (19.5%). The proportion of *N. farcinica* increased significantly over time from 13% in 2010 to 27.6% in 2014. Linezolid, amikacin, trimethoprim-sulfamethoxazole, minocycline, and imipenem were the most frequently identified active antibiotics with, respectively, 0% (0/734), 2.9% (21/730), 5.4% (40/734), 9.4% (69/734) and 19.5% (143/732) of isolates not susceptible. *N. farcinica* was frequently not susceptible to cefotaxime (118/148, 79.7% of the isolates), but only about 5% of *N. cyriacigeorgica* and *N. abscessus* complex isolates were not susceptible to cefotaxime.

Conclusions. In this first epidemiological study of *Nocardia* isolated from human samples in France, *N. farcinica* was the species most frequently identified and its prevalence increased over time.

INTRODUCTION

Nocardia species are Gram-positive filamentous bacteria found in a wide range of natural environments including decaying vegetation, soil and water [1, 2]. The use of molecular microbiology, including amplification and sequencing of the 16S RNA, *hsp65* and other genes, has led to the description of more than 100 species, so far [1, 3]. *Nocardia* can be responsible for severe opportunistic infections in immunocompromised patients and patients with chronic bronchopulmonary diseases [1, 4-6]. Infection follows bacterial inhalation or, less frequently, direct inoculation through the skin and can lead to pneumonia, brain abscesses and/or skin/soft-tissue infections [1].

The ideal initial treatment for nocardiosis should cover a broad-range of species with adequate antibiotic concentration in all involved organs [7]. However, defining the optimal treatment is difficult because of the lack of comparative and prospective clinical data. As a consequence, most antibiotic regimens currently proposed rely on microbiological data, including species identification and antimicrobial susceptibility testing (AST) [8-10]. Notably, each *Nocardia* species has a specific antibiotic susceptibility pattern [8-11]. Obtaining reliable species identification using molecular methods is therefore essential to guide initial antibiotic treatment.

We retrospectively collected data from a 6-year period (2010-2015) from a French laboratory dedicated to *Nocardia* (Observatoire Français des Nocardioses [OFN]). Our primary objective was to describe species distribution and AST results. Secondary objectives were to study temporal trends in species distribution, AST results and geographic distribution of *Nocardia* in France over the 6-year period.

METHODS

Study design, settings and inclusion criteria

We retrospectively reviewed results for isolates sent to the OFN for *Nocardia* testing between January 2010 and December 2015. The OFN is a French laboratory that has specialised in *Nocardia* and other *Actinomycetes* since 1999 [12]. French microbiology laboratories (from continental France and overseas territories) can send biological samples or bacterial isolates for molecular identification and/or AST; isolates from neighbouring countries are also analysed. The isolate density for each region of France was defined as the number of *Nocardia* isolates sent to our centre per year for 100,000 population of that region; population data for 2016 were used, obtained from the Institut national de la statistique et des études économiques (INSEE) [13].

Isolates were included in the present study if they fulfilled the following criteria: *i)* molecular-based confirmation that the strain belonged to the genus *Nocardia* (see below); *ii)* the strain had been isolated from a human clinical sample; *iii)* species identification had been performed by molecular biology (see below).

Variables

The following variables were recorded: demographic data (age and patient sex), year and geographic location of the isolated strain, type of clinical sample from which the *Nocardia* strain had been isolated (sputum, bronchial aspirate, bronchoalveolar lavage, pleural fluid, protected-specimen brush, cerebrospinal fluid, abscess fluid, organ biopsy, blood cultures), species identified and need to amplify *hsp65*, results of the AST. Continuous variables are presented as means (± standard deviation) or medians (range). Categorical variables are presented as numbers and frequencies.

Microbiology

Since 1999, the OFN uses a standardised protocol for microbiological analyses of *Nocardia*:

i) confirmation that the bacterial strain belongs to the genus *Nocardia* using a 16S-based *Nocardia* polymerase chain reaction (PCR) performed directly on the bacterial colony on an agar plate [14];

ii) species identification obtained by amplification and sequencing of a fragment of ~600 base pairs (bp) of the gene coding for the 16S ribosomal RNA (16S rRNA) using PCR [15]. The sequence obtained in this second step is compared to those stored in GenBank using blast alignment software (http://www.ncbi.nlm.nih.gov/blast) and

the BIBI (Bio Informatic Bacteria Identification tool: https://umr5558-bibiserv.univ-lyon1.fr/lebibi/lebibi.cgi) [16]. Identification at the species level requires 99.6% sequence similarity with the type strain of a single species. If more than 1 sequence in the database has more than 99% similarity, identification is made at the level of the complex. For *N. abscessus* complex or *N. transvalensis* complex, a 440 bp fragment of the *hsp65* gene is amplified and sequenced to obtain species identification [17]. Isolates with sequence similarities < 99% are identified as *Nocardia* spp..

iii) AST is performed using the disk diffusion method on cation-adjusted Mueller-Hinton (CA-MH) agar plates. Inoculums are prepared according to the Clinical and Laboratory Standards Institute (CLSI) standard M24-A2 [18, 19]. The antibiotic disks used are described in **Supplementary Table 1**. Results are read after 72 h of culture. For each antibiotic disk, the diameter of the inhibition zone is recorded and compared to thresholds **(Supplementary Table 1) [20]**. A "non-susceptible" isolate is defined as being resistant or intermediate. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Nocardia asteroides* ATCC 19247T were used as quality control organisms.

For trimethoprim-sulfamethoxazole, the plates are read at 80% of growth inhibition. If the inhibition zone is < 10 mm, an E-test strip is performed on a CA-MH agar plate [21]. If the trimethoprim-sulfamethoxazole minimum inhibitory concentration (MIC) obtained on the E-test strip is ≤ 2/38 µg/mL, the isolate is considered susceptible; if the MIC is ≥ 4/76 µg/mL, the strain is considered resistant [18].

Ethical aspects

This study was approved by the Comité de Protection des Personnes (CPP) Ile-de-France I Ethical board (CPPIDF1-2015-octobre-DAP 33), the CCTIRS (Comité consultatif sur le traitement de l'information en matière de recherche dans le domaine de la santé, file 16-355 approved May 19, 2016) and the CNIL (Comité National Informatique et Liberté).

RESULTS

Description of the isolates collected

During the study period (2010-2015), 793/823 *Nocardia* isolates met our entry criteria and were included in the analysis. Most isolates originated from France (696/793, 88%) (**Table 1**). The median patient age at sampling was 66 [4-90] years. Most of the *Nocardia* isolates were isolated from the lungs (427/793, 53.8%) or from subcutaneous abscesses or skin (156/793, 19.7%) (**Table 1**).

The most frequent species identified were *N. farcinica* (20.2%), *N. abscessus* complex (19.9%), *N. nova* complex (19.5%) and *N. cyriacigeorgica* (12.9%) (**Table 2**). *N. farcinica* was the most frequently isolated species in blood cultures and brain abscesses/cerebrospinal fluid: 21/39 (54%) and 19/43 (44.2%), respectively (**Supplementary table 2**). To obtain species identification for the *N. abscessus* and *N. transvalensis* complexes, *hsp65* amplification and sequencing was required for 179/793 isolates (22.6%).

Antibiotic susceptibility test results

AST was performed for 736 of the *Nocardia* isolates (92.8%). Linezolid, amikacin, trimethoprim-sulfamethoxazole, minocycline and imipenem were the antibiotics most frequently identified as being active against *Nocardia* with, respectively, 0%, 2.9%, 5.4%, 9.4% and 19.5% of isolates being non-susceptible (**Table 3**). For the *N. abscessus* complex, cefotaxime and ceftriaxone were the β-lactam antibiotics most frequently identified as being active (less than 3% of isolates were not susceptible), followed by meropenem and imipenem (7.3 and 11.8% of non-susceptible isolates, respectively) (**Table 3**). Conversely, *N. farcinica* isolates were frequently not susceptible to cefotaxime and ceftriaxone (~80% of the isolates) and meropenem (73% of isolates) but were frequently susceptible to amoxicillin/clavulanic acid or imipenem, with 20.1% and 23% of non-susceptible isolates, respectively. *N. nova* was more frequently not susceptible to amoxicillin-clavulanate (91.6%) than to amoxicillin (23.1%) and was infrequently not susceptible to imipenem (0.7%) and cefotaxime (20.3%). *N. cyriacigeorgica* was frequently not susceptible to amoxicillin-clavulanate (90.5%) and amoxicillin (87.2%) but was infrequently not susceptible to cefotaxime (7.4%) and imipenem (10.5%). *N. brasiliensis* was frequently not susceptible to amoxicillin (66.7%) and imipenem (85.1%) and amoxicillin-clavulanate was the most frequently active β-lactam (8.3% of *N. brasiliensis* isolates were not susceptible).

For carbapenem antibiotics, *N. farcinica* and *N. cyriacigeorgica* isolates were more frequently not susceptible to meropenem than to imipenem. Conversely, *N. transvalensis* complex and *N. brasiliensis* were more frequently not susceptible to imipenem than to meropenem. Non-susceptibility to different carbapenems was equally frequent for *N. abscessus* and *N. nova* complexes.

Less than 3% of the isolates were not susceptible to amikacin, with the notable exception of *N. transvalensis* complex in which 30.6% (15/49) were not susceptible. For gentamicin and tobramycin, more than 90% of *N. farcinica* and *N. transvalensis* complex isolates were not susceptible; 80% of *N. nova* complex isolates were not susceptible to tobramycin (**Table 3**).

Less than 10% of isolates were not susceptible to minocycline, but some species had higher rates of non-susceptibility, e.g., *N. cyriacigeorgica* (18.9%), *N. farcinica* (12.8%) and *N. brasiliensis* (12.5%).

Temporal trends

Between 2010 and 2013, the proportion of isolates identified as *N. farcinica* increased from 13% to 28.0%, decreasing thereafter to 19.3% in 2015 (overall Chi-square test, p=0.038) **(Figure 1**).

The frequency of isolates that were not susceptible to amikacin or trimethoprim-sulfamethoxazole remained stable between 2010 and 2015 at less than 9% for both drugs (**Supplementary Figure 1**).

Geographic distribution of *Nocardia* species

Among *Nocardia* isolates from France, isolate density was greater in Occitanie (the southernmost region of continental France), New Caledonia, Reunion and Guadeloupe than in other regions, with densities of 0.46, 0.42, 0.39 and 0.29 isolates/year/100,000 population, respectively (**Figure 2**). In continental France, isolate density was greater in the south east of the country than in other areas.

DISCUSSION

In this retrospective study, we analysed data from a French microbiology laboratory dedicated to the study of aerobic *Actinomycetes*. Among the 793 *Nocardia* isolates included, *N. farcinica*, *N. abscessus* complex, *N. nova* complex and *N. cyriacigeorgica* were the most frequently identified species, representing about three quarters of all isolates. In our sample, linezolid, amikacin and trimethoprim-sulfamethoxazole were the antibiotics that were most frequently identified as being active, each with less than 9% of isolates being non-susceptible.

The distribution of species we observed was different from that reported in recent studies from Spain (1119 isolates) and the United States of America (1299 isolates) in which *N. cyriacigeorgica* and *N. nova* complex were most frequently identified [9, 10]. Because a 16S rRNA +/- *hsp65* molecular identification strategy was used in Spain and in the present report, it is unlikely that species misclassification could explain these discrepancies. Of note, our isolates of *N. farcinica*, the most frequently identified species in our study, were not part of an outbreak. One possible explanation for the differences in species distribution is that climatic conditions may have an impact on the epidemiology of *Nocardia*, as suggested by the observation that *N. brasiliensis* is predominantly isolated in tropical or subtropical regions [1]. Furthermore, higher isolate density was observed in the South of France and overseas territories than in other regions, suggesting that environmental conditions may also increase the spread of *Nocardia*. The high densities observed in Reunion and New Caledonia also raise the possibility of an underlying genetic condition that may favour this opportunistic condition, such as alveolar proteinosis [28].

A phylogenetic analysis revealed that the 16S rRNA gene was not sufficient to obtain precise identification for some *Nocardia* complexes, such as *abscessus* or *transvalensis* [22]. Conversely, the 16S rRNA gene had sufficient discriminatory power for isolates belonging to the *N. nova* complex, *N. farcinica*, *N. cyriacigeorgica* or *brasiliensis* species [9]. Although our pragmatic approach (16S rRNA +/- *hsp65*) appears attractive for routine purposes, recent studies have demonstrated that the analysis of concatenated sequences of several genes, such as multilocus sequence analysis (MLSA), is likely to be more reliable for phylogenetic purposes [22]. In the near future, whole genome sequencing may also help to decipher *Nocardia* phylogeny.

Among the 736 *Nocardia* isolates that underwent AST, the antibiotics most frequently identified as active against *Nocardia* were linezolid, amikacin, trimethoprim-sulfamethoxazole, minocycline and imipenem. The main challenge in the field of *Nocardia* AST is the lack of data correlating AST results with clinical

outcomes. As a consequence, technical guidelines are derived from *in vitro* studies assessing the reproducibility and the technical pitfalls of each method. In our study, AST was performed by disk diffusion, even though the CLSI has stated that broth microdilution is the technical gold-standard for *Nocardia* [18]. However, the CLSI also acknowledged that broth microdilution may have limitations, including false-resistant results for ceftriaxone against *N. brasiliensis* or for imipenem against *N. farcinica* [18]. Sulphonamide testing with broth microdilution is also challenging and requires disk diffusion for confirmation if a resistant strain is identified [18]. Another pitfall of broth microdilution is its poor interlaboratory reproducibility, especially for some antibiotic/species combinations, such as ceftriaxone against *N. cyriacigeorgica* and *N. wallacei* or sulphonamides against *N. farcinica* and *N. wallacei* [23]. Furthermore, testing several agents with broth microdilution is time-consuming, especially given the lack of available commercial plates dedicated to *Nocardia* testing in Europe. As a consequence, alternative methods are required to routinely perform AST for *Nocardia*. First described in 1973 by Michael C. Bach and co-workers, antibiotic disk diffusion was further developed and analysed by Richard Wallace's group [24, 25]. In 1997, results from a direct comparison of broth microdilution, antibiotic disk diffusion, agar dilution, E-test and the BACTEC radiometric method on 26 *Nocardia* isolates were published [19]. There was 100% agreement of antibiotic disk diffusion results with the study "gold standard" for amikacin, erythromycin, imipenem, minocycline and trimethoprim-sulfamethoxazole; conversely, 80 to 88% agreement was observed for ampicillin, amoxicillin-clavulanic acid, ceftriaxone and ciprofloxacin. Overall agreement for the disk diffusion method was 95.7%. Of note, this study included only a small number (9/27) of the antibiotics we tested. The discrepancies regarding β-lactam results may be related to inducible β-lactamase whose production can vary depending on the experimental conditions. The comparison of results from large-scale microbiological studies performed with broth microdilution [8, 9], E-tests [10] and disk diffusion (present study) highlights the difficulties in interpreting *Nocardia* AST results (see a comprehensive overview of these data on **Supplementary table 3)** [8-10]. Our results using disk diffusion to assess susceptibility of cefotaxime/ceftriaxone against *N. cyriacigeorgica* (low frequency of resistance) and against *N. farcinica* (high frequency of resistance) were similar to those from studies using other methods [8-10]. Conversely, there were significant discrepancies regarding imipenem susceptibility against *N. farcinica* with 67% of isolates identified as resistant by broth microdilution [9], compared to 4% by E-test [10] and 23% by disk diffusion. These results raise the question of stability issues for imipenem in broth microdilution and stress the need to interpret AST results according to the method used [26].

To our knowledge, this study is the first in which *Nocardia* epidemiology has been assessed over time and across geographical regions in France. The proportions of most species remained stable over time with the notable exception of *N. farcinica* for which the proportion increased significantly between 2010 and 2014. We recently observed that *N. farcinica* was more common among solid organ transplant (SOT) recipients [4, 27]. As SOT now appears to be one of the leading and increasingly common conditions favouring nocardiosis, this increase in the proportion of *N. farcinica* may be explained by an increase in the proportion of cases of post-SOT nocardiosis.

The main strengths of our study include the large number of isolates reliably identified at the species level using standardised methods performed by the same technical team with the same guidelines during the study period. However, there are also several limitations, including the fact that reporting nocardiosis is not mandatory in France. Thus, the isolates sent to the OFN may reflect a particular interest of the local clinical microbiology laboratory or a lack of knowledge in this field. Nevertheless, although we have described the largest sample of *Nocardia* isolates in France so far, it is likely that we missed some cases.

In conclusion, we provide results from the first large-scale epidemiological study of *Nocardia* in France. *N. farcinica* was the most frequently identified species and identification rates increased over time. AST performed by disk diffusion appears to be a reliable method for routine testing of *Nocardia*.

Transparency declaration

Conflict of interest: None

Funding: David Lebeaux was supported by the following grant: Bourse Junior 2015–Société de Pathologie Infectieuse de Langue Française (SPILF).

Acknowledgments: The authors would like to thank Koucila Chekhrit and Clémence Bruyere for their help in data collection and Dr Karen Pickett for her editorial suggestions.

Maps were prepared with Articque, Make your Map, freely available on the internet and d-maps.com.

The authors would like to thank M.C. Gaps and J.M. Ballec for their conscientious scientific assistance.

REFERENCES

[1] Brown-Elliott BA, Brown JM, Conville PS, Wallace RJ, Jr. Clinical and laboratory features of the *Nocardia* spp. based on current molecular taxonomy. Clin Microbiol Rev **2006**; 19(2): 259-82.

[2] Saubolle MA, Sussland D. Nocardiosis: review of clinical and laboratory experience. J Clin Microbiol **2003**; 41(10): 4497-501.

[3] Euzeby JP. List of Bacterial Names with Standing in Nomenclature: a folder available on the Internet (http://www.bacterio.net/). Int J Syst Bacteriol **1997**; 47(2): 590-2.

[4] Coussement J, Lebeaux D, van Delden C, Guillot H, Freund R, Marbus S, et al. *Nocardia* infection in solid organ transplant recipients: a multicenter European case-control study. Clin Infect Dis **2016**; 63(3): 338-45.

[5] Minero MV, M. M, Cercenado E, Rabadan PM, Bouza E, Munoz P. Nocardiosis at the turn of the century. Medicine (Baltimore) **2009**; 88(4): 250-61.

[6] Rodriguez-Nava V, Durupt S, Chyderiotis S, Freydiere AM, Karsenty J, de Montclos M, et al. A French multicentric study and review of pulmonary *Nocardia* spp. in cystic fibrosis patients. Med Microbiol Immunol **2015**; 204(4): 493-504.

[7] Lebeaux D, Morelon E, Suarez F, Lanternier F, Scemla A, Frange P, et al. Nocardiosis in transplant recipients. Eur J Clin Microbiol Infect Dis **2014**; 33(5): 689-702.

[8] Larruskain J, Idigoras P, Marimon JM, Perez-Trallero E. Susceptibility of 186 *Nocardia* sp. isolates to 20 antimicrobial agents. Antimicrob Agents Chemother **2011**; 55(6): 2995-8.

[9] Schlaberg R, Fisher MA, Hanson KE. Susceptibility profiles of *Nocardia* isolates based on current taxonomy. Antimicrob Agents Chemother **2014**; 58(2): 795-800.

[10] Valdezate S, Garrido N, Carrasco G, Medina-Pascual MJ, Villalon P, Navarro AM, et al. Epidemiology and susceptibility to antimicrobial agents of the main *Nocardia* species in Spain. J Antimicrob Chemother **2016**.

[11] Wallace RJ, Jr., Steele LC, Sumter G, Smith JM. Antimicrobial susceptibility patterns of *Nocardia asteroides*. Antimicrob Agents Chemother **1988**; 32(12): 1776-9.

[12] Boiron P, Provost F, Chevrier G, Dupont B. Review of nocardial infections in France 1987 to 1990. Eur J Clin Microbiol Infect Dis **1992**; 11(8): 709-14.

[13] Rapport INSEE 2016 - https://www.insee.fr/fr/statistiques/2554860 - documentation.

[14] Couble A, Rodriguez-Nava V, de Montclos MP, Boiron P, Laurent F. Direct detection of *Nocardia* spp. in clinical samples by a rapid molecular method. J Clin Microbiol **2005**; 43(4): 1921-4.

[15] Cloud JL, Conville PS, Croft A, Harmsen D, Witebsky FG, Carroll KC. Evaluation of partial 16S ribosomal DNA sequencing for identification of *Nocardia* species by using the MicroSeq 500 system with an expanded database. J Clin Microbiol **2004**; 42(2): 578-84.

[16] Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res **1997**; 25(17): 3389-402.

[17] Rodriguez-Nava V, Couble A, Devulder G, Flandrois JP, Boiron P, Laurent F. Use of PCR-restriction enzyme pattern analysis and sequencing database for *hsp65* gene-based identification of *Nocardia* species. J Clin Microbiol **2006**; 44(2): 536-46.

[18] CLSI. *Susceptibility Testing of Mycobacteria, Nocardiae and Other Aerobic Actinomycetes; Approved Standard-Second Edition*. CLSI document M24-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.

[19] Ambaye A, Kohner PC, Wollan PC, Roberts KL, Roberts GD, Cockerill FR, 3rd. Comparison of agar dilution, broth microdilution, disk diffusion, E-test, and BACTEC radiometric methods for antimicrobial susceptibility testing of clinical isolates of the *Nocardia asteroides* complex. J Clin Microbiol **1997**; 35(4): 847-52.

[20] https://www.resapath.anses.fr/resapath_uploadfiles/files/Documents/2013_CASFM.pdf.

[21] Lowman W, Aithma N. Antimicrobial susceptibility testing and profiling of *Nocardia* species and other aerobic actinomycetes from South Africa: comparative evaluation of broth microdilution *versus* the Etest. J Clin Microbiol **2010**; 48(12): 4534-40.

[22] McTaggart LR, Richardson SE, Witkowska M, Zhang SX. Phylogeny and identification of *Nocardia* species on the basis of multilocus sequence analysis. J Clin Microbiol **2010**; 48(12): 4525-33.

[23] Conville PS, Brown-Elliott BA, Wallace RJ, Jr., Witebsky FG, Koziol D, Hall GS, et al. Multisite reproducibility of the broth microdilution method for susceptibility testing of *Nocardia* species. J Clin Microbiol **2012**; 50(4): 1270-80.

[24] Bach MC, Gold O, Finland M. Activity of minocycline against *Nocardia asteroides*: comparison with tetracycline in agar-dilution and standard disc-diffusion tests and with sulfadiazine in an experimental infection of mice. J Lab Clin Med **1973**; 81(5): 787-93.

[25] Wallace RJ, Jr., Septimus EJ, Musher DM, Martin RR. Disk diffusion susceptibility testing of *Nocardia* species. J Infect Dis **1977**; 135(4): 568-76.

[26] Viaene E, Chanteux H, Servais H, Mingeot-Leclercq MP, Tulkens PM. Comparative stability studies of antipseudomonal beta-lactams for potential administration through portable elastomeric pumps (home therapy for cystic fibrosis patients) and motor-operated syringes (intensive care units). Antimicrob Agents Chemother **2002**; 46(8): 2327-32.

[27] Lebeaux D, Freund R, van Delden C, Guillot H, Marbus SD, Matignon M, et al. Outcome and Treatment of Nocardiosis After Solid Organ Transplantation: New Insights From a European Study. Clin Infect Dis **2017**; 64(10): 1396-405.

[28] Hadchouel A, Wieland T, Griese M, Baruffini E, Lorenz-Depiereux B, Enaud L, et al. Biallelic Mutations of Methionyl-tRNA Synthetase Cause a Specific Type of Pulmonary Alveolar Proteinosis Prevalent on Reunion Island. Am J Hum Genet **2015**; 96(5): 826-31.

TABLES AND FIGURES

Table 1. Characteristics of 793 *Nocardia* isolates analysed at the Observatoire Français des Nocardioses (2010-2015)

Characteristics	793 *Nocardia* isolates
Patient demographical data	
Age at *Nocardia* sampling (years) (median, range) n= 693	66 [4-90]
Male (n, %) n=743	432 (58.1)
Year of sampling (n, %)	
2010	123 (15.5)
2011	130 (16.4)
2012	127 (16.0)
2013	132 (16.6)
2014	141 (17.8)
2015	140 (17.7)
Country of origin (n, %) n=791	
France	696 (88.0)
Belgium	31 (3.9)
Switzerland	28 (3.5)
Netherlands	23 (2.9)
Portugal	7 (0.9)
Spain	2 (0.3)
Monaco	1 (0.1)
Lebanon	1 (0.1)
Luxembourg	1 (0.1)
Tunisia	1 (0.1)
Site and method of sampling (n, %)	
Lung	427 (53.8)
Sputum	156 (19.7)
Bronchoalveolar lavage	112 (14.1)
Bronchial aspirate	99 (12.5)
Lung biopsy	20 (2.5)
Pleural fluid	20 (2.5)
Bronchial biopsy	1 (0.1)
Protected-specimen brush	1 (0.1)
Unspecified	18 (2.3)
Cutaneous biopsy or subcutaneous abscess sample	156 (19.7)
Blood culture	39 (4.9)
Brain abscess	37 (4.7)
Joint fluid	18 (2.3)

Cerebrospinal fluid	6 (0.8)
Liver biopsy	2 (0.3)
Bone biopsy	2 (0.3)
Corneal abscess	1 (0.1)
Lymph node	1 (0.1)
Pericardial biopsy	1 (0.1)
Other abscess fluid	1 (0.1)
Unknown	102 (12.9)

Table 2. **Identified species for the 793 *Nocardia* isolates.**

Nocardia species	793 *Nocardia* isolates
N. farcinica	160 (20.2)
N. abscessus complex	158 (19.9)
N. abscessus sensu stricto	59
N. abscessus/N.araoensis-like	15
N. abscessus/N.arthritidis-like	6
N. arthritidis	9
N. arthritidis/gamkensis/exalbida-like	30
N. beijingensis	22
N. asiatica	4
N. testacae	2
N. abscessus complex	11
N. nova complex	155 (19.5)
N. nova sensu stricto	144
N. veterana	11
N. cyriacigeorgica	102 (12.9)
N. brasiliensis	55 (6.9)
N. transvalensis complex	51 (6.4)
N. wallacei	39
N. transvalensis sensu stricto	11
N. blacklockiae	1
N. otitidiscaviarum	13 (1.6)
N. brevicatena/paucivorans complex	11 (1.4)
N. pseudobrasiliensis	8 (1.0)
N. flavorosea	7 (0.9)
N. cerradoensis	6 (0.8)
N. mexicana	4 (0.5)
N. carnea	3 (0.4)
N. jiangxiensis	3 (0.4)
N. goodfellowii	2 (0.3)
N. asteroides	2 (0.3)
N. puris	2 (0.3)
N. higoensis	2 (0.3)
N. mikamii	2 (0.3)
N. pneumoniae	2 (0.3)
N. coubleae	1 (0.1)
N. altamirensis	1 (0.1)
N. elegans	1 (0.1)
N. neocaledoniensis	1 (0.1)

N. concava	1 (0.1)
N. rhamnosiphila	1 (0.1)
N. takedensis	1 (0.1)
N. uniformis	1 (0.1)
N. vinacea	1 (0.1)
Nocardia spp.	36 (4.5)

Table 3. Results of antibiotic susceptibility testing among 736 *Nocardia* isolates according to species

Antibiotic, n (%) of non-susceptible* isolates	All *Nocardia* isolates. (n=736)	*Nocardia abscessus* complex (n=152)	*Nocardia farcinica* (n=149)	*Nocardia nova* complex (n=145)	*Nocardia cyriacigeorgica* (n=95)	*Nocardia transvalensis* complex (n=49)	*Nocardia brasiliensis* (n=48)
Amoxicillin	394 (53.8)	37 (24.3)	137 (91.9)	33 (23.1)	82 (87.2)	29 (59.2)	32 (66.7)
Amoxicillin-clavulanic acid	365 (49.7)	40 (26.3)	30 (20.1)	131 (91.6)	86 (90.5)	6 (12.2)	4 (8.3)
Ticarcillin-clavulanic acid	372 (53.8)	42 (27.6)	58 (43.6)	131 (94.2)	82 (93.2)	5 (10.9)	5 (10.9)
Piperacillin-tazobactam	457 (66.0)	39 (26.7)	129 (97.0)	103 (74.1)	83 (93.3)	31 (67.4)	17 (37.0)
Imipenem	143 (19.5)	18 (11.8)	34 (23.0)	1 (0.7)	10 (10.5)	18 (36.7)	40 (85.1)
Meropenem	227 (31.2)	11 (7.3)	108 (73.0)	6 (4.2)	41 (43.6)	8 (16.7)	28 (58.3)
Doripenem	119 (29.1)	8 (9.4)	68 (68.7)	4 (5.1)	11 (25.0)	2 (6.9)	16 (76.2)
Ertapenem	303 (42.7)	17 (11.6)	123 (89.8)	18 (12.7)	59 (62.8)	23 (48.9)	31 (64.6)
Cefotaxime	193 (26.4)	4 (2.7)	118 (79.7)	29 (20.3)	7 (7.4)	0 (0.0)	11 (22.9)
Ceftriaxone	209 (28.5)	4 (2.6)	120 (80.5)	42 (29.4)	4 (4.2)	2 (4.1)	15 (31.3)
Cefepime	281 (38.9)	12 (7.9)	134 (91.2)	33 (23.4)	33 (35.1)	8 (17.0)	27 (56.3)
Cefuroxime	98 (25.0)	3 (3.8)	64 (69.6)	7 (9.1)	1 (2.2)	1 (3.6)	9 (42.9)
Pristinamycin	82 (94.3)	22 (88.0)	19 (95.0)	13 (100.0)	7 (87.5)	7 (100.0)	2 (100.0)
Gentamicin	224 (30.7)	2 (1.3)	136 (91.9)	22 (15.5)	1 (1.1)	45 (95.7)	1 (2.1)
Tobramycin	333 (45.6)	2 (1.3)	143 (96.6)	114 (79.7)	1 (1.1)	46 (95.8)	0 (0.0)
Amikacin	21 (2.9)	1 (0.7)	2 (1.4)	0 (0.0)	1 (1.1)	15 (30.6)	0 (0.0)
Minocycline	69 (9.4)	2 (1.3)	19 (12.8)	10 (6.9)	18 (18.9)	2 (4.1)	6 (12.5)
Doxycycline	176 (42.8)	4 (4.8)	63 (63.6)	54 (67.5)	10 (21.7)	14 (48.3)	14 (66.7)
Tigecycline	202 (27.9)	11 (7.4)	66 (45.2)	66 (46.5)	15 (15.8)	23 (47.9)	2 (4.3)
Erythromycin	463 (64.1)	88 (58.7)	143 (97.3)	6 (4.3)	90 (95.7)	44 (91.7)	41 (85.4)
Linezolid	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Vancomycin	465 (63.8)	91 (60.7)	46 (31.1)	108 (76.1)	81 (85.3)	43 (87.8)	45 (93.8)

Trimethoprim	530 (76.5)	63 (43.4)	125 (94.0)	137 (97.9)	47 (52.8)	46 (100.0)	43 (93.5)
Trimethoprim-sulfamethoxazole	40 (5.4)	2 (1.3)	6 (4.0)	12 (8.3)	3 (3.2)	6 (12.2)	2 (4.2)
Ciprofloxacin	520 (71.4)	131 (86.8)	62 (41.9)	139 (97.9)	95 (100.0)	5 (10.2)	42 (89.4)
Levofloxacin	409 (56.2)	105 (69.5)	41 (27.7)	128 (90.1)	81 (86.2)	3 (6.1)	18 (37.5)
Moxifloxacin	264 (36.4)	73 (48.7)	14 (9.5)	90 (63.8)	58 (61.7)	3 (6.4)	1 (2.0)
Rifampin	505 (73.0)	88 (60.7)	121 (91.0)	106 (76.3)	56 (62.9)	43 (93.5)	40 (87.0)

*Non-susceptible trains were defined as resistant or intermediate.

Other species who underwent AST were: *N. otitidiscaviarum* (n=11), *N. brevicatena/paucivorans* complex (n=9), *N. pseudobrasiliensis* (n=8), *N. flavorosea* (n=7), *N. cerradoensis* (n=4), *N. mexicana* (n=4), *N. carnea* (n=3), *N. jiangxiensis* (n=2), *N. asteroides* (n=2), *N. higoensis* (n=2), *N. mikamii* (n=2), *N. pneumoniae* (n=2), *N. goodfellowii* (n=1), *N. puris* (n=1), *N. coubleae* (n=1), *N. altamirensis* (n=1), *N. elegans* (n=1), *N. neocaledoniensis* (n=1), *N. concava* (n=1), *N. rhamnosiphila* (n=1), *N. takedensis* (n=1), *N. uniformis* (n=1), *N. vinacea* (n=1), *Nocardia* spp. (n=31).

FIGURES

Figure 1. Proportions of species (%) from 2010 to 2015. The proportion of *N. farcinica* increased significantly over time (overall Chi-square test, p=0.038). Horizontal bars indicate pairwise comparisons between 2010 (13%) and 2013 (28%), 2014 (27.6%) and 2015 (19.3%), all with $p < 0.05$, Fisher's exact test.

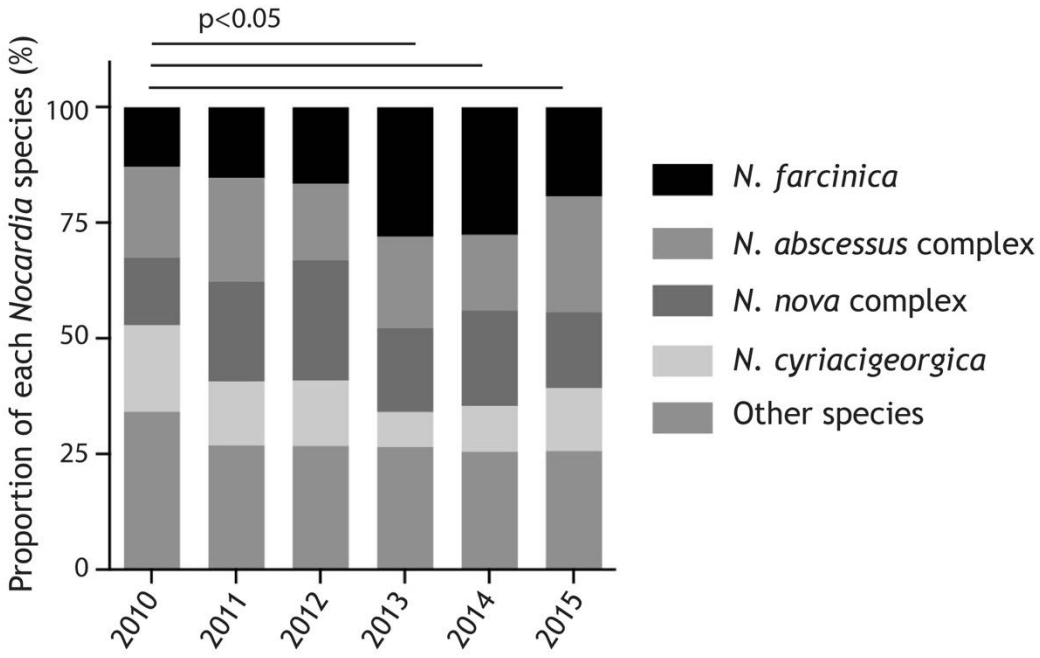

Figure 2. **Geographical distribution of the 696 *Nocardia* isolates originating from France (continental France and overseas territory).** For each region, two values are depicted: first, the number of isolates collected during the study period and then the "isolate density" defined as the number of isolates per year for 100,000 population. The colour code represents the number of isolates/year/100,000 population for each region. One isolate for Mayotte Island and one isolate for French Polynesia are not depicted. Occitanie is the southernmost region of continental France and is coloured in black.