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ORIGINAL INVESTIGATION

A French multicentric study and review of pulmonary *Nocardia* spp. in cystic fibrosis patients

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Abstract Some bacterial species recovered from the airways of cystic fibrosis (CF) patients are indisputably associated with lung infections, whereas the clinical relevance of others, such as *Nocardia* spp., remains unclear. Sixteen French CF cases of colonization/infection with *Nocardia* spp. were reviewed in order to evaluate the epidemiology, the clinical impact and the potential treatment of these bacteria, and results were compared to those of the literature. Five *Nocardia* species were identified, *Nocardia cyriacigeorgica* being the major species (50 % of cases). At first isolation, *Nocardia* was the sole pathogen recovered in six patients. Seven patients presented pulmonary exacerbation. For 12 patients, antimicrobial treatment against *Nocardia* was started immediately, mainly based on cotrimoxazole (6 of the 12 cases). In this study, we highlight the heterogeneity of the clinical management of *Nocardia* spp. in CF. Guidelines for the clinical management of *Nocardia* infections in CF patients are proposed.

Keywords *Nocardia* · Cystic fibrosis · Lung infections · Antimicrobial therapy · Guidelines

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Introduction

Pulmonary infections in cystic fibrosis (CF) have been recognized as the major cause of morbidity and mortality, leading to the irreversible failure of lung functions [1]. A succession of pathogens is classically described, starting with *Haemophilus influenzae* and *Staphylococcus aureus*, progressing to *Stenotrophomonas maltophilia, Pseudomonas aeruginosa* and *Burkholderia* spp. [2]. However, CF lungs are ecological niches with complex and atypical microbiota. The virulence of some unusual species in CF has not been clearly demonstrated, and their clinical interest remains unclear [3, 4]. The gram-positive branching and filamentous rods *Nocardia* spp. belong to these "uncertain" CF pathogens [5].

Nocardia spp. are ubiquitous worldwide-spread actinobacteria that can be isolated from soil, composting vegetation and water. After inhalation or direct inoculation, these bacteria can cause localized or disseminated human diseases (nocardiosis) with tissue destructions, abscesses, systemic disorders and metastatic lesions [5–7]. Among all these infections, 60–80 % are associated with pulmonary nocardiosis [8]. Symptoms of nocardiosis are unspecific and can be fever, thorax pain, increased sputum and night sweats. X-ray shows multiple lung infiltrates that are sometimes associated with necrosis [7]. Common risk factors for nocardiosis include immunosuppression, malignancies and severe lung disease (chronic obstructive pulmonary disease, bronchiectasis, emphysema…) [6, 9–12]. Though CF is associated with complex immunologic and metabolic disorders, lung damage as well as corticosteroid or transplantation-related immunosuppression, *Nocardia* spp. are rarely isolated in the sputum of CF patients, as suggested by the few number of cases reported in the literature. Indeed, Barrio et al. [5] described the isolation of *Nocardia* spp. in only 9 out of 387 patients (2.3 %) followed in 6 CF units in Spain. This low incidence of nocardiosis among CF patients may be explained by the fact that current laboratory protocols do not include specific processing of CF specimens for *Nocardia* isolation. Moreover, CF patients frequently receive several antimicrobial drugs against major pathogens that could prevent the development of *Nocardia* spp. in lungs either directly or by modifying the pulmonary ecosystem. Lastly, the CF population is mainly constituted of children, whereas nocardiosis is described preferentially in adults [6, 8]. However, the increasing life expectancy of CF patients could imply a potential increase in the incidence of *Nocardia* infections and supports the need of improving our knowledge of their pathogenicity in this clinical setting.

When *Nocardia* spp are isolated from CF sputum, their clinical significance and the corresponding patient management remain unclear. Determining the difference between *Nocardia* infections and colonizations is challenging since the availability of study cohorts is limited due to the low incidence of nocardiosis in CF. Moreover, nocardiosis can be under-diagnosed since the pulmonary symptoms are unspecific. In the literature, both infections and colonizations are described. Lastly, *Nocardia* spp. are rarely the only microorganism present in CF lungs [6, 7].

The objective of the present report was to describe in 3 French CF centers the clinical and microbiological features of 16 CF patients with sputum culture positive for *Nocardia* spp. in order to (1) analyze the impact of such bacteria on the lung status and (2) review the clinical practices. Finally, we propose guidelines for the clinical management of *Nocardia* infections in CF patients.

Patients and methods

Patients

CF patients from 3 French CF centers (Lyon, Montpellier and Toulouse) were included in our retrospective study. All had at least one sputum culture positive for *Nocardia* spp. between 2001 and 2011. For each patient, we recorded age, sex, clinical status, pulmonary function tests (PFT) results (forced vital capacity—FVC and forced expiratory volume in 1 s—FEV_1), body mass index (BMI) at the time of *Nocardia* isolation and antimicrobial treatments (Table 1). Patients were classified as "patient with acute pulmonary exacerbation" as defined by Fuchs et al. [13] when they presented a combination of at least 4 of the following symptoms: change in sputum, new or increased

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Table 1 Patients' characteristics

Patient	Sex	Age of diagnosis	Genotype	Age at first isolation of *Nocardia* (years)	Antimicrobial treatment within the three previous months	Acute exacerbation	Clinical symptoms	PFT		BMI
								FVC (%)	FEV1 (%)	
1	F	1 month	N1303K/T2640Del	13	amc/clox/azt	No	Recent bronchial sounds Recent opacity of the right superior lobe	101	107	17.8
2	M	25 years	W1282X/V232D	28	azt	No	Diffuse bronchial sounds Weight loss (5 kg)	100	87	24.9
3	M	Birth	ΔF508/ΔF508	9	tm/cs/azt	No	None	100	110	19.5
4	F	Birth	ΔF508/ΔF508	13	cpdx/clox	No	None	91	97	15.2
5	M	3 years	ΔF508/ΔF508	15	mno/azt/pt	Yes	Purulent sputum Dyspnea at physical activity Purulent rhinoconjunctivitis Asthenia Fever and night sweats	90	80	17.8
6	F	2 months	ΔF508/W1282X	11	cro/tm	No	None	87	91	14.7
7	M	Birth	N1303K/W1282X	6	caz/tm/sxt	Yes	Symptoms associated with an infection with Aspergillus	68	50	12.8
8	M	Birth	ΔF508/ΔF508	18	pt/sxt/cs/azt	No	Cough Increased sputum production Modified TDM	79	73	18.8
9	M	17 years	ΔF508/3,849 + 10kbC>T	26	caz/tm/sxt/cip/cs/azt/mno	No (but with frequent exacerbations)	Cough Increased sputum production Asthenia Fever Inflammatory polyarthralgia	103	84	19.7
10	F	68 *years*	Unknown	67	none	Yes	Cough Increased sputum production	109.2	89.3	15.6
11	F	Birth	ΔF508/ΔF508	16	caz/tm	No	Cough Increased sputum production	111	81	21.6
12	M	10 years	ΔF508/S1251N	12	tm/cip	Yes	Cough Sputum	90	83	16.1

Table 1 continued

Patient	Sex	Age of diagnosis	Genotype	Age at first isolation of *Nocardia* (years)	Antimicrobial treatment within the three previous months	Acute exacerbation	Clinical symptoms	PFT		BMI
								FVC (%)	FEV1 (%)	
13	M	3 months	ΔF508/ΔF508	20	sxt/tm/tem	Yes	Thoracic pain	50	37	20.3
14	M	4 months	ΔF508/R334W	20	amc	Yes	Cough Sputum Weight loss Recurrent pancreatitis	57	46	18
15	M	5 months	ΔF508/G542X	11	cip/tm	Yes	Increased sputum production and cough	90	88	20.5
16	M	10 years	Unknown	78	Unknown	No	Cough	93	69	22.9

Patients from Toulouse, Lyon and Montpellier are separated by dashes. Patients with acute expectoration are italicized

PFT pulmonary function tests, *FVC* forced vital capacity, *FEV1* forced expiratory volume in 1 s, *BMI* body mass index; *amc* amoxicillin + clavulanic acid, *azt* azithromycine, *caz* ceftazidime, *cip* ciprofloxacin, *clox* cloxacillin, *cpdx* cefpodoxime, *cro* ceftriaxone, *cs* colistin, *mno* minocycline, *pt* pristinamycin, *sxt* trimethoprim–sulfamethoxazole (cotrimoxazole), *tm* tobramycin, *tem* temocillin

hemoptysis, increased cough, increased dyspnea, malaise or fatigue or lethargy, temperature above 38 °C, anorexia or weight loss, sinus pain and tenderness, change in sinus discharge, change in physical examination of the chest, decrease in the pulmonary function by 10 % or more from a previously recorded value, or radiographic change indicative of pulmonary infection.

Microbiological data

In the hospital microbiological laboratories which analyzed the patients' sputa, *Nocardia* strains were isolated by classical sputa analysis (culture during 5 days at 37 °C on various media, not specific of the *Nocardia* species). The associated pathogens and the total number of *Nocardia* positive sputa were recovered. At least one *Nocardia* isolate from each patient was sent to the French Observatory for Nocardiosis (FON, Lyon, France) which is a center specialized in the identification and antimicrobial susceptibility tests of *Nocardia* sp. Isolates from several sputa were sent for six patients (Table 2).

Nocardia identification was performed by the sequencing of both 16S rRNA gene and *hsp65* gene as recommended for the separation between phylogenetically close species [14, 15]. A 600-bp fragment of 16S rRNA gene and a 440-bp fragment of *hsp65* gene were amplified by PCR according to the protocols established by Rodriguez-Nava et al. [16]. After sequencing, the sequence analysis was performed with BLAST software (nucleotide–nucleotide BLAST http://www.blast.ncbi.nlm.nih.gov/Blast.cgi) [17]. The identification at species level was done according to the similarity percentage with the reference species present in GenBank. The minimal similarity percentage requested for each identification was 99 %.

The antibiotic susceptibility testing was performed and analyzed using disk diffusion method on cation-supplemented Mueller-Hinton medium according to the CLSI standards M24-A2 and CA-SFM guidelines [18, 19]. The antibiotic disks used were amoxicillin (25 µg), amoxicillin–clavulanic acid (20/10 µg), ceftriaxone (30 µg), cefotaxime (30 µg), cefepime (30 µg), ciprofloxacin (5 µg), linezolid (30 µg), trimethoprim–sulfamethoxazole (1.25/23.75 µg), amikacin (30 µg), gentamicin (15 µg), tobramycin (10 µg), imipenem (10 µg), minocycline (30 µg), vancomycin (30 µg) and erythromycin (15 µg) (Bio-Rad, Marnes-la-Coquette, France).

Results

Eighteen patients were described as having sputum culture positive for *Nocardia* spp between 2001 and 2011. Two patients were excluded because of the lack of clinical and

Table 2 Microbiological data and antimicrobial treatment

Patient	Species	Number of positive specimens (duration of *Nocardia* isolation)	Number of isolates analyzed by FON	Associated pathogens at first *Nocardia* isolation	Treatment initiation	Antibiotics drugs
1	*N. cyriacigeorgica*	2 (/1 mo)	1	None	Immediately	imp/am (15 d) clox (1 mo)
2	*N. farcinica*	2 (/2 mo)	1	*S. aureus* *S. maltophilia*	Immediately	sxt (3 w)
3	*N. farcinica*	2 (/2 mo)	1	*S. maltophilia*	Immediately	sxt (duration not known)
4	*N. abscessus*	2 (/7 mo)	2	None	Immediately	amc (15 d)
	N. veterana	1	1			
5	*N. cyriacigeorgica*	1	1	*S. aureus* *H. influenzae* Candida	Immediately	sxt (3 mo)
6	*N. cyriacigeorgica*	2 (/1 mo)	1	None	Immediately	amc (15 d) sxt (10 d) sxt/cro/tm (8 d) sxt (3 mo)
7	*N. cyriacigeorgica*	1	1	*S. aureus* *P. aeruginosa* *S. pneumonia* *Aspergillus*	No treatment	
8	*N. abscessus*	3 (/15 mo)	2	*S. aureus* *P. aeruginosa* *Candida* *Aspergillus*	1 year after primo-infection	imp/an (15 d) sxt/cro (6 mo) sxt (6 mo)
9	*N. wallacei*	7 (≥6 years)	3	*S. aureus* *P. aeruginosa* *Aspergillus*	3 year after primo-infection	cro/cip (15 d) amc (2 mo) cro/an (3 mo)
10	*N. cyriacigeorgica*	6 (/38 mo)	4	None	Immediately	lzd (3 mo)
11	*N. cyriacigeorgica*	1	1	*S. maltophilia* *P. aeruginosa*	Immediately	lzd (15 d)
12	*N. cyriacigeorgica*	5 (/31 mo)	2	None	Immediately	sxt/caz (15 d)
13	Nocardia spp	1	ND	*S. aureus* *S. maltophilia* *H. influenzae* *Aspergillus*	Immediately	sxt/cip (21 d) + tm IV (7 d) then nebulized tm
14	*N. farcinica*	11 (/47 mo)	3	*S. aureus* *H. alvei*	Immediately	lzd (15 d)
15	*N. cyriacigeorgica*	1	1	*S. aureus* *S. maltophilia* *Aspergillus*	Immediately	sxt/amc (3 mo)
16	*N. abscessus*	2 (/16 mo)	1	None	No treatment	

Patients from Toulouse, Lyon and Montpellier were separated by dashes. Patients with acute exacerbation are italicized

am ampicillin, *amc* amoxicillin + clavulanic acid, *an* amikacin, *caz* ceftazidime, *cip* ciprofloxacin, *clox* cloxacillin, *cro* ceftriaxone, *imp* imipenem, *lzd* linezolid, *mno* minocycline, *sxt* trimethoprim–sulfamethoxazole (cotrimoxazole), *tm* tobramycin

d day, *mo* month

ND Not determined. The strain could not be isolated from the specimen. Just a direct detection by PCR could be performed

[a] Resistant in a second strain isolated 1 month later

[b] Resistant since the second strain isolated 3 months after the first one

biological data. The remaining 16 CF patients (5 females and 11 males) had consistent recorded clinical data and were included in our study. Most of them carried a homozygote Δ508 mutation (6/16) or a heterozygote Δ508 mutation (5/16). Other mutations were W1282X/V232D, N1303K/W1282X and N1303K/T2640Del, whereas the CFTR genotypes of the two oldest patients (patients 10 and 16) were unknown. As expected, most of the patients (14/16 patients) were treated by oral, IV or nebulized antibiotics during the 3 months before *Nocardia* isolation. No patient was transplanted (Table 1).

The age of patients at first *Nocardia* isolation varied from 6 to 78 years (mean age = 22.68 years; median age = 15.5 years) with nine patients (56.3 %) younger than 18 years.

Concerning the clinical status at the first *Nocardia* isolation, 7 patients showed an acute pulmonary exacerbation (patients 5, 7, 10 and patients 12–15) (Table 1). Three of these seven patients (patients 7, 13, 14) had low spirometry results (FEV1 < 70 % pred) and 3 (patients 7, 10 and 12) had a BMI lower than 16.5. The nine patients without acute exacerbation had spirometry results close to (patient 6) or superior to 90 %. Only two of them (patients 4 and 6) had a BMI lower than 16.5. However, 5 of these 9 patients without such exacerbation (patients 1, 2, 8, 9, 11) presented at least 2 systemic (fever, loss of weight, asthenia) or respiratory changes (cough, increased sputum, modified chest X-ray) (Table 1). Notably, patient 9 was described with frequent exacerbations even though this was not the case at the time of the first *Nocardia* isolation. Moreover, patient 6 developed a pulmonary infection 2 weeks after the *Nocardia* isolation. Finally, patients 8 and 9 were described as having functional lung degradation between 1 and 3 years later, whereas *Nocardia* was still isolated from sputa (data not shown).

At the time of the first isolation, *Nocardia* was the sole pathogen in 6 patients, two patients with pulmonary exacerbation (patients 10 and 12), one patient (patient 1) with several recent respiratory changes (apparition of bronchial sounds and lobar opacity) and three patients with no more than one relevant symptom (patients 4, 6 and 16). In the 10 other cases, *Nocardia* was associated with other pathogens (*S. aureus* in 8 patients, *S. maltophilia* in 5 patients, *P. aeruginosa* in 4 patients and *Aspergillus* in 5 patients). Particularly, patient 7 was described as having bronchopulmonary aspergillosis.

Partial sequencing of 16S rRNA and *hsp*65 genes identified 5 species of *Nocardia*: *N. cyriacigeorgica* ($n = 8$), *N. farcinica* ($n = 3$), *N. abscessus* ($n = 3$), *N. wallacei* ($n = 1$) and *N. veterana* ($n = 1$). Patient 4 was co-colonized by two *Nocardia* species: *N. abscessus* and *N. veterana* (Table 2). For patient 13, the isolate sent to FON was overgrown by another organism and could only be identified at the genus level (Table 2). Most of the strains were isolated from at least two sputa (11/16 patients), and for 6 patients, more than twice (3–11 sputa). Colonization of the CF tract lasted from 1 month to up to 47 months except for patient 9 who still grows *Nocardia* in 2014 (Table 2).

Antimicrobial susceptibility tests were performed for 15 patients (Table 3). For patients for whom FON received more than one strain, the results of these tests were compared. Notably, for patient 4, the first and third isolated strains were susceptible to the trimethoprim–sulfamethoxazole association (cotrimoxazole), whereas the second (isolated 1 month before the third one) was resistant. For patient 9, the first strain was susceptible to imipenem, whereas the two others, isolated, respectively, 3 and 5 months later, were resistant. For patients 8, 10, 12 and 14, the different strains did not present any relevant differences.

Twelve patients received an antimicrobial treatment against *Nocardia* immediately after the first *Nocardia* isolation, including six of the seven patients presenting an exacerbation, whereas two others (patients 8 and 9) were treated 1 and 3 years later, respectively. Patient 7 was treated by itraconazole for aspergillosis infection and patient 16 did not receive any specific treatment. The main first-line antimicrobial drug was oral cotrimoxazole in monotherapy (3 patients) or in association with ceftazidime, amoxicillin–clavulanic acid or ciprofloxacin and tobramycin (3 patients). For two patients (patients 6 and 8), cotrimoxazole was used as second-line treatment. The duration of cotrimoxazole treatment varied between 15 days and 3 months. The other antimicrobial drugs used were linezolid, amoxicillin–clavulanic acid, penems, cephalosporins, aminoglycosides and fluoroquinolones.

Among the six patients with exacerbations and immediately treated specifically for a *Nocardia* infection (as explained before, patient 7 was treated for aspergillosis), a significant improvement of respiratory symptoms and spirometry was noted for 4 patients: patients 5 and 14 for who *Nocardia* was isolated with other pathogens and patients 10 and 12 for who no other pathogen than *Nocardia* was identified. In the same way, this clinical improvement was also noted for the two patients (patients 8 and 9) treated later, i.e., when a deterioration of the lung function was observed.

Discussion

Due to the increasing life expectancy of CF patients, as well as the use of specific procedures for CF sputum analysis and the development of new methods for bacterial identification, many uncommon bacteria are identified in CF patient sputa [3]. As for several of these opportunistic pathogens, the clinical impact of the *Nocardia* species in CF

Table 3 Antimicrobial susceptibility

Patient	Species	Antimicrobial susceptibility	Antimicrobial resistance
1	*N. cyriacigeorgica*	an, cro, ctx, fep, gen, ipm, lzd, tm, sxt	amc, amx, cip, e, mno, va
2	*N. farcinica*	an, amc, cip, ipm, lzd, mno, sxt	amx, cro, ctx, e, fep, gen, tm, va
3	*N. farcinica*	an, amc, cip, cro, ipm, lzd, va	amx, ctx, e, fep, gen, mno, sxt, tm
———	———	———	———
4	*N. abscessus*	an, amc, amx, cro, ctx, (e)[a], fep, gen, ipm, lzd, mno, tm, (sxt)[a], (va)[a]	cip
	N. veterana	an, amc, amx, cro, ctx, gen, ipm, lzd, mno, sxt	cip, e, fep, tm, va
5	*N. cyriacigeorgica*	an, amx, cro, ctx, fep, gen, ipm, lzd, mno, tm, sxt, va	amc, cip, e
6	*N. cyriacigeorgica*	an, amc, cro, ctx, fep, gen, ipm, lzd, mno, sxt, tm	amx, cip, e, va
7	*N. cyriacigeorgica*	an, cro, ctx, gen, ipm, lzd, mno, sxt, tm	amc, amx, cip, e, fep, va
———	———	———	———
8	*N. abscessus*	an, amc, amx, cro, ctx, (e)[a], fep, gen, ipm, lzd, mno, sxt, tm	cip, (va)[c]
9	*N. wallacei*	amc, cip, cro, ctx, (ipm)[b], lzd, mno	an, amx, e, fep, gen, sxt, tm, va
———	———	———	———
10	*N. cyriacigeorgica*	an, cro, ctx, gen, ipm, lzd, sxt, tm	amc, amx, cip, e, fep, mno, va
11	*N. cyriacigeorgica*	an, cro, ctx, fep, gen, ipm, lzd, mno, sxt, tm, va	amc, amx, cip, e
12	*N. cyriacigeorgica*	an, cro, ctx, fep, gen, ipm, lzd, sxt, tm	amc, amx, cip, e, mno, va
13	*Nocardia spp*	unknown[ND]	unknown[ND]
14	*N. farcinica*	an, cip, ipm, lzd, mno, sxt, (va)[d]	amc, amx, cro, ctx, e, fep, gen, tm
15	*N. cyriacigeorgica*	an, cro, ctx, gen, ipm, lzd, sxt, tm, va	amc, amx, cip, e, fep, mno,
16	*N. abscessus*	an, amc, amx, cro, ctx, e, fep, gen, ipm, lzd, mno, sxt, tm, va	cip

Patients from Toulouse, Lyon and Montpellier were separated by dashes. Patients with acute exacerbation are italicized

amc amoxicillin + clavulanic acid, *amx* amoxicillin, *an* amikacin, *cip* ciprofloxacin, *cro* ceftriaxone, *ctx* cefotaxime, *e* erythromycin, *fep* cefepime, *gen* gentamicin, *imp* imipenem, *lzd* linezolid, *mno* minocycline, *sxt* trimethoprim–sulfamethoxazole, *tm* tobramycin, *va* vancomycin

ND Not determined. The strain could not be isolated from the specimen. Just a direct detection by PCR could be performed

[a] Resistant in a second strain isolated 1 month later

[b] Resistant since the second strain isolated 3 months after the first one

[c] Susceptible in a second strain isolated 1 year later

[d] Resistant since the third strain isolated 3 years after the first one

remains unclear, notably because of the small number of reported cases. We describe a retrospective French multicentric study with 16 cases of CF patients harboring *Nocardia* sp. To our knowledge, our study reports, together with one from Thorn et al. [10], the largest number of *Nocardia* CF cases described in the literature (Table 4).

Patients included in our study were young adults or children except for two cases. This is coherent with the life expectancy of CF patients and with the literature: Only 7 out of the 37 previously described patients (18.9 %) were older than 25 years (Table 4). However, these results are unexpected considering general data on nocardiosis. Indeed, *Nocardia* infections are known to be rare in the general pediatric population, and advanced age is known to be a risk factor for nocardiosis. Therefore, if *Nocardia* species isolated in CF patient lungs are linked to infections, these results could suggest that CF must be considered as an additional risk factor beside the age. If not, the high level of *Nocardia* species' identification in young CF patients may be due to the high number of bacterial sputa analyses that are performed for these child than to the others leading to an increase in the identification of their colonizations.

Concerning the clinical status at first *Nocardia* isolation, nine patients presented no exacerbation of their pulmonary status suggesting a colonization of the lungs by *Nocardia* (Table 1), as already proposed by some authors [5, 20]. However, 5 of these 9 patients had notable systemic or respiratory symptoms. For these 5 patients as well as for the 6 with an acute exacerbation (total of patients = 11), the clinical status could be explained by the presence of *Nocardia* spp. as a real pathogen. This is particularly true when a clinical improvement was observed after specific *Nocardia* treatment or when *Nocardia* is the only microorganism isolated from sputa. However, in case of multimicrobial sputa, it is difficult to know which pathogen exacerbates the lung symptoms, and the role of each bacterium in the lung pathophysiology remains uncertain. Due to their known virulence in CF, the major pathogens *P. aeruginosa* and *S. aureus* must be treated in priority. However, as described by Aravantagi et al. [11], some patients receiving

Table 4 *Nocardia* cases in patients with CF described in the literature

	Nocardia spp. (number of isolates)	Number of cases and sex	Age (years)	Symptoms	Treatment	Infection (I) or colonization (C)
Taj-Aldeen [35]	*N. otitidiscaviarum*	1 F	17	Severe pneumonia	cla/sxt + mxf	I
Aravantagi [11]	*N. transvalensis*	1 M	19	Acute exacerbation	sxt/lzd (15 d)	I
					sxt (15 d) + lzd (7 d)	
Barrio [5]	*N. spp* (3)			Six patients with symptoms (Three acute exacerbations/Three exacerbated cough)	6 sxt (10 d to 6 mo)	C rather than I
	N. asteroides (2)	9 (8 F, 1 M)			3 mno (1 mo to 6 mo)	
	N. farcinica (1)		10–28		1 imp/an (14 d)	
	N. transvalensis (1)				1 mer/tm (14 d)	
	N. elegans (1)			3 patients without symptoms	2 no treatment	
	N. asiatica (1)					
Beucher [7]	*N. farcinica*	1 M	20	Multiple symptoms (starting with a pneumothorax)	sxt/caz + amk (1 mo) sxt (6 mo)	I
Bittar [29]	*N. farcinica*	1 M	15	Multiple symptoms	cip/stx/an (21 d) sxt/an (6 mo)	I
Dasgupta [36]	*N. farcinica*	1 F	31	Multiple symptoms	sxt > 6 mo	I
Kohn [12]	*N. asteroides*	1 M	5.5	Multiple symptoms	sxt 6 mo	I
Lumb [20]	*N. asteroides*	2 M	41	Exacerbation	sxt (1 mo)	C
			17		sxt (9 mo) but not immediately	
		1 F	18		sxt (4 mo) tic/tm sxt (>15 mo)	
Pablo [37]	*N. asteroides*	1 M	6	Exacerbation	sxt (6 w)	I
Petersen [6]	*N. farcinica*	1 M	8	Exacerbation	sxt (2 mo)	C or I
Thorn [10]	*N. asteroides* (13)					
	N. farcinica (1)	6 F			13 sxt	
	N. transvalensis (1)	11 M	2–53	–	3 mno	C rather than I
	N. cyriacigeorgica (1)				1stx/mno/dox	
	N. otitidiscaviarum (1)					
Total	*N. spp* (3)	37 patients	5.5–53			
	N. asteroides (20)					
	N. farcinica (6)	17 F	Mean: 20.88			
	N. transvalensis (3)	20 M				
	N. cyriacigeorgica (1)		7 patients > 25 years			
	N. elegans (1)					
	N. asiatica (1)					
	N. otitidiscaviarum (2)					

an amikacin, *caz* ceftazidine, *cip* ciprofloxacin, *cla* clarithromycin, *dox* doxycycline, *imp* imipenem, *lzd* linezolid, *mer* meropenem, *mno* minocycline, *mxf* moxifloxacin, *sxt* trimethoprim–sulfamethoxazole, *tic* ticarcillin, *tm* tobramycin, *va* vancomycin

d day, *w* week, *mo* month

F female, *M* male

adapted treatment against major pathogens presented lung deterioration, whereas the clinical improvement was obvious with *Nocardia* therapy. Therefore, when *Nocardia* is the sole pathogen isolated in the lung or when the first-line antibiotics fail to restore the lung functions, diagnosis of nocardiosis must be proposed. Moreover, even if *Nocardia* is not always linked to pulmonary nocardiosis, its presence could enhance lung damages induced by other bacteria

[21]. It should be noticed that even if Beucher et al. [7] suggested that the association *Nocardia*/*Pseudomonas* seems to be correlated to increased symptoms, in our study, only four sputa contained *P. aeruginosa* and only two of them were associated with acute exacerbations.

There are currently no guidelines for when (immediately or not) and how to treat the *Nocardia* infections, and this lack of recommendations might explain the discrepancies among the treatments observed in the literature and within our study [10, 22, 23]. Concerning our cohort, most of the patients were treated immediately after isolation of *Nocardia*. For two patients, the therapy was performed a long time after the isolation when the persistence of *Nocardia* colonization was confirmed by the multiple positive samples (no spontaneous eradication was observed) and when the clinical status of these patients got worse. Very short treatment durations were observed (only 2 weeks with amoxicillin–clavulanic acid for patient 4) reflecting the doubt of the clinician to treat and to treat for a long time, especially when no symptoms were noticed (Tables 2, 3). Indeed, antibiotic therapy against *Nocardia* is often associated with adverse effects [10]. As an example, cotrimoxazole could induce rash, bone marrow suppression and urinary lithiasis [23]. Moreover, CF patients receive multiple treatments and the addition of supplemental treatments should be kept to the strict minimum. However, as suggested by Khon et al., short-term treatments might expose patients to nocardiosis relapses [11]. Several patients presented repeated sputum cultures with *Nocardia* species even after the instauration of an appropriate antimicrobial treatment and a clinical improvement. This suggests that the *Nocardia* eradication could take time as earlier indicated and that a prolonged therapy must be necessary in addition to a long-term follow-up of sputum culture. Surprisingly, even with this treatment and the improvement of the symptoms, patient 9 is still colonized by *Nocardia*. In regard to the literature, treatments from 6 to 12 months are recommended for serious lung infections or in patients with strong immunosuppression. If symptoms are few, treatment could be reduced to 1–3 months [23]. If intravenous therapy is initially used, it could be switched to oral therapy after the clinical improvement [23]. Currently, the use of cotrimoxazole as first-line treatment is reported in the literature as in our study (Table 4) [23]. However, few resistant strains are described, as those of patients 3 and 9 (*N. farcinica* and *N. wallacei*, respectively) [24]. Surprisingly, in the case 3, despite this resistance, the patient presented a good evolution with a long-term cotrimoxazole-based treatment. This drug could be used in association with another antibiotic such as an aminoglycoside or a cephalosporin based on the severity of the infection and the *Nocardia* antimicrobial susceptibility pattern. However, there are some *Nocardia* species that are resistant to these agents. For example, *N. transvalensis* and *N. wallacei* are usually resistant to aminoglycoside antibiotics [25, 26], and this was confirmed by our patient 9. In addition, *N. farcinica* is most frequently resistant to cephalosporin antibiotics [26], as was the case for patients 2, 3 and 14 (Tables 3 and 4). Carbapenem, minocycline and linezolid have also been shown to be efficient against *Nocardia* spp. and were also used in our study [8, 22].

Various *Nocardia* species have been described in CF: *N. asteroides complex* (20 cases), *N. farcinica* (6 cases), *N. transvalensis* (3 cases), *N. elegans* (1 case), *N. asiatica* (1 case), *N. cyriacigeorgica* (1 case) and *N. otitidiscaviarum* (2 cases) (Table 4).

The current molecular methods of identification have led to the separation of *N. asteroides complex* mainly into two different species: *N. cyriacigeorgica* and *N. abscessus*. Previously published case reports concerning *N. asteroides complex* used other methods, and therefore, the exact incidence of these two species in these reports remains unknown. If some of the *N. asteroides* strains described in the literature were in fact *N. cyriacigeorgica*, our results would be consistent with those published before. Moreover, most of the species found in these patients have already been identified as the etiologic agents most commonly found in France according to the FON [*N. farcinica* (26 %), *N. nova* (20 %), *N. abscessus* (18 %) and *N. cyriacigeorgica* (12 %)]. Finally, this study is also the first one to describe the species *N. wallacei* and *N. veterana* in CF. Due to the long-term infection with *N. wallacei*, it could be interesting to investigate the adaptability of this pathogen in CF lungs. Moreover, a coinfection case was described in patient 4. This coinfection highlights the need to correctly differentiate the *Nocardia* morphotypes in the culture in order to give a correct diagnosis and a more appropriate treatment. Indeed, species may present different antibiotic susceptibility patterns.

For several patients, we could observe the evolution of the susceptibility of the isolated strain. In most cases, no modifications in these susceptibility patterns were observed, except for two cases where resistance appeared. Notably, for patient 9 who was first treated by imipenem, a resistance to this drug appeared and the antimicrobial therapy was adapted thereafter.

Pathogenic *Nocardia* species can grow in different environments [27]. Therefore, activities which imply a direct and repeated exposure with soil (agricultural activities, gardening, etc.) must be performed with the appropriate protection in order to avoid direct inoculations and the contact with aerosols, or simply avoided in the case of high-risk population (chronic obstructive pulmonary disease, bronchiectasis, long-term corticosteroid therapy, organ transplant, cancer, HIV, etc.) [28]. Such recommendations might be extended to the CF population or at least, since it could

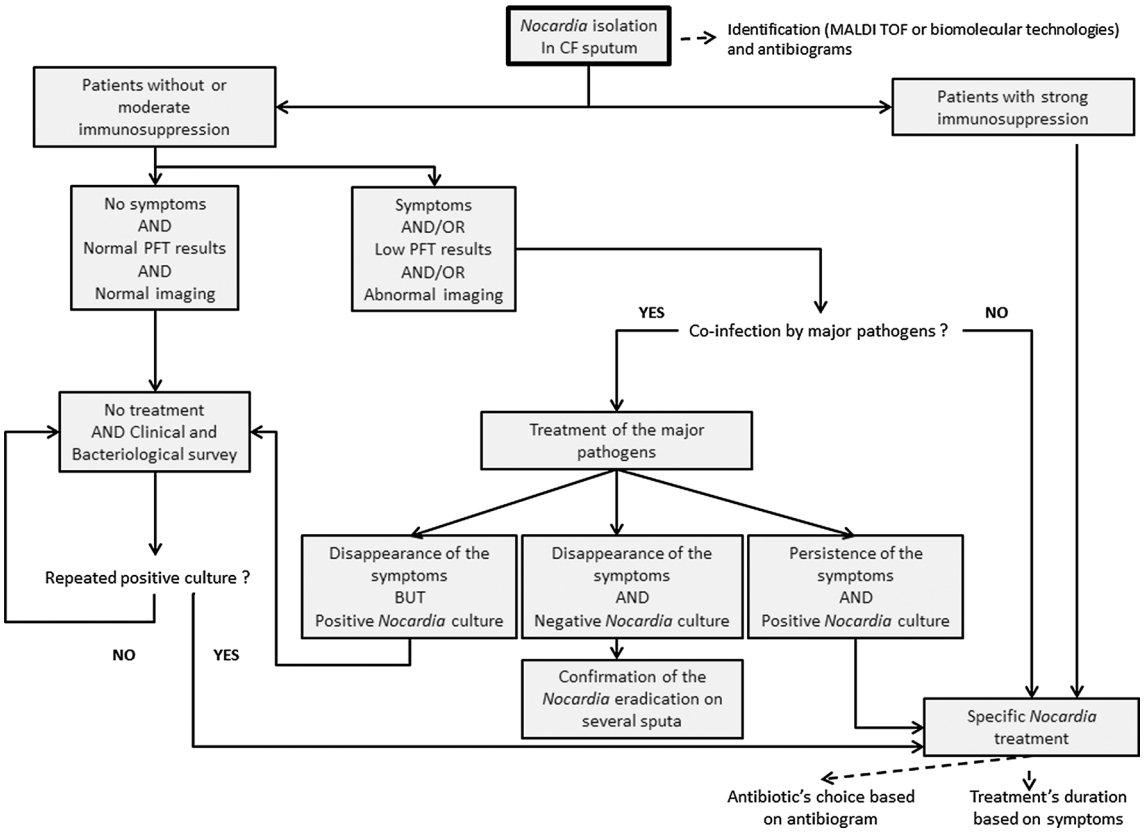

Fig. 1 Proposed guidelines for the clinical management of *Nocardia* infections in CF patients

be constraining for children, to the immunocompromised CF patients.

According to the potential implication of *Nocardia* spp. in CF lung pathologies, we suggest the following therapeutic strategy for patient colonized by *Nocardia* spp. (Fig. 1). (1) When bronchopulmonary symptoms are present or increased and/or the PFT results show decreased lung function and/or the imaging shows opacity/nodules, antibiotics should first target and treat usual pathogens isolated in patients' sputum. In these cases, *Nocardia* treatment can be postponed. Thereafter, if the clinical status remains unchanged and if the *Nocardia* is still present, patients should be treated specifically for *Nocardia*. If major pathogens are absent, *Nocardia* treatment must be done immediately. (2) If the patient has no symptoms, good lung function and no changes in chest X-ray since the precedent visit, the treatment could be delayed while maintaining a close clinical and microbiological monitoring. To make possible this targeted survey of the *Nocardia* colonization in lungs, colonized untreated patients and patients with *Nocardia* history should be clearly identified by the clinicians and the bacteriologists. If a change occurs in the clinical status of the patient, the treatment must be initiated if no other pathogen is incriminated. Similarly, a prolonged isolation of *Nocardia* in sputa must be taken into account and potentially treated despite stable clinical status. Finally, patients with particular clinical status (lung transplantation or strong immunosuppressive regimen or may be repeated corticoid treatment) must be treated because such conditions are known to induce nocardiosis [29].

For all the CF patients, *Nocardia* species should be carefully searched for in sputum even if the patient has received, as in our study, a previous cotrimoxazole treatment. Indeed, several studies showed that this antibiotic does not protect from nocardiosis in immunocompromised patients particularly with malignancies or HIV infection [30, 31]. Analyzing another type of sample could be discussed since as indicated by Khon and Conrad [12], a bronchoalveolar lavage allowed the isolation of a *Nocardia* strain that was missed in sputum. Samples must be inoculated on rich media such as blood or chocolate agar plates or on selective media such as BCYE (buffered charcoal yeast extract medium) and observed at least 7 days at 37 °C and, ideally, at 30 °C [32]. Some *Nocardia* spp. with slow growth (*N. abscessus*, *N. nova*, *N. elegans*, *N. wallacei*…) could be searched for by incubating the culture for at least 20 days [8]. Molecular or proteomic techniques such as matrix-assisted laser desorption ionization-time of flight

(MALDI-TOF) mass spectrometry technology could help in the nocardial identification after cultivation and sometimes a detection of *Nocardia* directly from the human clinical samples using specific molecular tools to help in the diagnosis [33, 34]. A follow-up of the antimicrobial susceptibility tests should be realized as soon as *Nocardia* is isolated, particularly in case of lack of clinical improvement under treatment, because some *Nocardia* species can be multidrug resistant.

When necessary, targeted *Nocardia* therapy (like cotrimoxazole) has to be initiated and should be continued based on the severity of the lung disease and the evolution of the antimicrobial susceptibility. Treatment duration less than 1 month must be proscribed because of its inefficiency and of the risk of nocardiosis relapse.

Conclusion

With the improving survival of patients with CF, the microbial spectrum described in the pulmonary system continues to evolve, implying a potential rise in the incidence of *Nocardia* and the need to improving our knowledge on their pathogenicity.

In this series of case reports, we have brought some evidence to consider *Nocardia* spp. as potential pathogens for certain CF patients and showed that the decision of antibiotic treatment at first isolation remains uncertain, while it should be considered for patients having signs of exacerbation. We therefore proposed guidelines to help clinicians in their therapeutic decisions.

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