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FULL PAPER

Nocardia infection over 5 years (2011-2015) in an Italian tertiary care hospital

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SUMMARY

This study was conducted reviewing clinical records of 14 patients affected by nocardiosis over 5 years in a tertiary care hospital. *Nocardia abscessus* was responsible for one third of infections, deviating significantly from the results reported by other epidemiological investigations and highlighting the key role of molecular identification tests. Indeed, a precise identification of species is crucial for the determination of antibiotic sensitivity patterns and, consequently, for the choice of antibiotic treatment. Noteworthy, 40% of isolates of *N. abscessus* (formerly *N. asteroides complex*) showed resistance to carbapenems, which are usually recommended for empirical therapy.

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INTRODUCTION

Nocardia spp. are aerobic, gram-positive, slow-growing bacteria, which are ubiquitous in the environment (soil organic material and water). This microorganism has the ability to cause localized or systemic disease both in humans and animals (Wilson *et al.,* 2012) (Valerio Minero *et al.,* 2009). In humans, accordingly to the route of transmission (direct inhalation or inoculation), *Nocardia* spp. infections are mainly localized in the lower respiratory tract and the soft tissue. Nevertheless, nocardiosis is mainly an opportunistic infection, which affects immunocompromised hosts in up to 60% of cases (Beaman *et al.,* 1994), causing severe, life-threatening disseminated infections (Saubolle *et al.,* 2003) (Ambrosioni *et al.,* 2010). Chronic obstructive pulmonary disease (COPD), chronic renal failure, alcoholism and diabetes mellitus are predisposing factors, as well as other cell-mediated immunodeficiencies, such as those related to prolonged steroid therapy, chemotherapy, HIV infection, hematologic malignancies, immunosuppressive treatment for solid organ or bone marrow transplantation and autoimmune diseases (Sorel *et al.,* 2010) (Beaman *et al.,* 1994) (Peleg *et al.,* 2007) (Castro *et al.,* 2007) (Filice *et al.,* 2005) (Valerio Minero *et al.,* 2009). Considering the introduction and spread of new, highly immunosuppressive drugs, *Nocardia* spp. infections will likely continue to emerge as important opportunistic diseases (Ambrosioni *et al.,* 2010) (Martinez *et al.,* 2008) (Munoz *et al.,* 2007) (Valerio Minero *et al.,* 2009) (Matulionyte *et al.,* 2004). As shown in the literature, the greater the degree of cell-mediated immunodeficiency, the higher the *Nocardia* spp. infection mortality, whose values range between 7% and 44% (Ambrosioni *et al.,* 2010) (Chedid *et al.,* 2007).

The microbiological diagnosis of nocardiosis, which has always been challenging, has recently improved. Indeed, conventional culture identification of *Nocardia* spp., based on phenotypic methods, lacks sufficient discriminatory power, has a long turnaround time and requires highly trained staff. Over the last years, the introduction of molecular methods, such as sequencing of the 16S rRNA gene, in routine diagnostic practice has allowed a more accurate and easier identification of *Nocardia* spp.. The availability of this technology better clarifies the taxonomy of *Nocardia* spp., which has an impact in clinical practice. As a matter of fact, as different *Nocardia* spp. have different profiles of antimicrobial susceptibility, a proper identification could have an essential impact in the choice of the treatment (Brown-Elliott *et al.,* 2006) (Roth *et al.,* 2003) (Woo *et al.,* 2008).

This 5-year retrospective study was carried out to focus on microbiological and clinical findings in patients with a confirmed infection due to *Nocardia* spp. in an Italian tertiary care hospital.

MATERIAL AND METHODS

This retrospective study was conducted reviewing clinical records of patients affected by nocardiosis, followed at the Verona University Hospital between January 2011 and December 2015.

Considering the lack of guidelines for nocardiosis, there is no an international agreement on the precise definition of such infections. We chose a conservative approach, considering as infected the patients who presented clinical signs/symptoms and radiological/laboratory findings

Key words:
Nocardia infection, Pulmonary nocardiosis, Cerebral nocardiosis, Opportunistic infection, Immunocompromised host.

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supporting the diagnosis and in whom *Nocardia* spp. was isolated from whichever biological specimen. Conversely, colonized subjects were defined as individuals showing no clinical, radiological or laboratory abnormalities and in whom *Nocardia* spp. was isolated from non-sterile biological specimens. Only infected patients were enrolled in the study.

We collected demographic characteristics, including age and sex, as well as underlying conditions, risk factors, clinical features, laboratory, radiological and microbiological investigations (identification of the species and antimicrobial susceptibility data).

Considering the antibiotic treatment, we collected data on both type and duration of treatment. In particular, we defined as genus-driven the target therapy administered immediately after the detection of the genus *Nocardia*, as species-driven the one started on the basis of species typing and antibiotic susceptibility testing, and as maintenance therapy the oral regimen given to patients once their clinical status had improved.

Regarding the patient's risk factors, we were specifically interested in assessing exposure to immunosuppressive drug, such as chemotherapeutic agents, calcineurin inhibitors, tumor necrosis factor-alpha inhibitors and corticosteroids. In order to assess the long-term outcome, patients were followed up to one year after the beginning of the treatment.

Antimicrobial susceptibility was determined using the broth microdilution method following the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (Clinical and Laboratory Standards Institute, 2003). Genotypic identification of *Nocardia* spp. was performed by the PCR analysis of 16S rRNA gene polymerase chain reaction (PCR) and sequencing (Clinical and Laboratory Standards Institute, 2003).

All statistical analyses were performed using STATA software, version 14.2 (College Station, TX: StataCorp LP). Continuous variables were expressed as means with standard deviations (SD) and medians with interquartile ranges (IQR). The percentage of patients in each category was calculated for categorical variables.

RESULTS

Between 2011 and 2015, *Nocardia* spp. was isolated in 26 individuals: 12 were considered colonized, and thus excluded, whereas 14 patients were included in the study. The male:female ratio was 9:5 and median age was 63 years (IQR, 46 - 82 years).

Among the study population (N=14), 13 subjects (92.8%) showed at least one underlying condition. Hematologic malignancies (n = 4, 28.57%) and autoimmune diseases (n = 4, 28.57%) were the more frequent, followed by solid malignancies (n = 2, 14.28%), diabetes mellitus (n = 2, 14.28%), chronic renal failure (n = 2, 14.28%), solid organ transplantation (n = 1, 7.14%), COPD (n = 1, 7.14%), trauma-related finger injury (n = 1, 7.14%) and alcoholism (n = 1, 7.14%).

Table 1 - *Individual characteristics of the 14 patients with invasive nocardiosis*

Patient	*Site of infection*	*Specimen*	*Nocardia species*	*Genus - driven target therapy*	*Species - driven target therapy*	*Maintenance therapy*	*Surgery*
1	Soft tissue	Subcutaneous abscess	*N. cyriacigeorgica*	TMP-SMX	TMP-SMX Ampicillin/sulbactam	Amoxicillin/ clavulanate	Drainage
2	Lung	Sputum	*N. abscessus*	Imipenem TMP-SMX	Ceftriaxone TMP-SMX	TMP-SMX	None
3	CNS	Brain biopsy	*N. abscessus*	Meropenem TMP-SMX	Meropenem Linezolid	TMP-SMX	Brain abscess aspiration
4	Disseminated (lung, CNS, soft tissue, bone, kidney)	Blood, subcutaneous abscess	*N. farcinica*	Meropenem Linezolid	Meropenem Linezolid	TMP-SMX	Brain abscess aspiration and drainage of subcutaneous abscesses
5	CNS	Brain biopsy	*N. farcinica*	Meropenem Linezolid	Meropenem Linezolid	TMP-SMX	None
6	Peritoneum	Peritoneal fluid	*N. farcinica*	Imipenem Linezolid	Imipenem Amikacin	Amoxicillin/ clavulanate	None
7	Lung	Bronchial aspirate	*N. abscessus*	Imipenem TMP-SMX	Imipenem Levofloxacin	TMP-SMX	None
8	Lung	Sputum	*N. farcinica*	Meropenem TMP-SMX	Ceftriaxone TMP-SMX	TMP-SMX	None
9	Lung	Bronchial aspirate	*N. cyriacigeorgica*	None	None	None	None
10	Lung	Bronchial aspirate	*N. cyriacigeorgica*	None	None	None	None
11	Lung	Bronchial aspirate	*N. cyriacigeorgica*	Meropenem TMP-SMX	Meropenem Ampicillin/sulbactam	Amoxicillin/ clavulanate	None
12	Lung	Bronchial aspirate	*N. abscessus*	TMP-SMX	TMP-SMX Meropenem	Levofloxacin	None
13	Lung	Bronchial aspirate	*N. cyriacigeorgica*	TMP-SMX Linezolid	TMP-SMX Ceftriaxone	TMP-SMX	None
14	Disseminated (lung, CNS)	Brain biopsy	*N. abscessus*	Ceftriaxone TMP-SMX	Ceftriaxone TMP-SMX Intrathecal amikacin	Minocycline	Brain abscess aspiration and implantation of Ommaya reservoir

Abbreviations: CNS, central nervous system; TMP-SMX, trimethoprim/sulfamethoxazole.

Immunosuppressive therapy was identified as the most common risk factor (n = 9, 64%): 7 patients had been receiving prednisone at a median dose per day of 25 mg (IQR, 5 - 50 mg) for a median of 6 months (IQR, 3 - 24 months) before developing nocardiosis. None of them was taking trimethoprim/sulfamethoxazole prophylaxis.

The individual characteristics of the enrolled patients are shown in *Table 1*.

Overall, the pleuropulmonary region was the most involved site (n = 10, 71.43%), followed by central nervous system (n = 4, 28.57%), skin and soft tissue (n = 2, 14.28%), kidney (n = 1, 7.14%) and peritoneum (n = 1, 7.14%). Disseminated disease, defined as the isolation of *Nocardia* from the bloodstream or multiple organ involvement, occurred in two (14.28%) cases. Within the disseminated disease group, both patients had lung and central nervous system involvement, whereas one had also kidney and soft tissue infections. Concomitant infections were diagnosed in only one subject, affected by a suspected pulmonary aspergillosis.

The clinical onset was heterogeneous, depending on the involved site. Nevertheless, half of the patients (n = 7) showed systemic symptoms, including fever, weight loss, malaise and night sweats. Respiratory symptoms, including cough (n = 6, 42.86%), production of sputum (n = 4, 28.57%), dyspnea (n = 2, 14.28%), haemoptysis (n = 1, 7.14%) and pleuritic chest pain (n = 1, 7.14%), were observed in 57.14% (n = 8) of study participants. Central nervous system nocardiosis presented with seizures (n = 2, 14.28%), expressive aphasia (n = 1, 7.14%) and changes in mental status (n = 1, 7.14%). Skin and soft tissue infection consisted of subcutaneous abscesses in both cases, limited to a trauma-related finger injury in the localized disease.

At the time of diagnosis, the mean and median leukocyte counts were 8587.27/mm^3 (SD, 5305.87/mm^3) and 8630/mm^3 (IQR, 4750 - 12390/mm^3), respectively; the mean and median C-reactive protein levels were 91.22 mg/L (SD, 76.22 mg/L) and 79 mg/L (IQR, 29.5 - 134 mg/L). CD4+ T lymphocytes counts were available in five patients only, displaying a mean and median values of 382.8/mm^3 (SD, 160.50/mm^3) and 405/mm^3 (IQR, 352 - 483/mm^3), respectively. Serum (1,3)-Beta-D-glucan (BG) assay was performed in seven patients, displaying a negative result in two subjects and a value above 400 pg/mL in the remaining five individuals.

Chest X-ray and computerized tomography (CT) findings included nodules (n = 3), cavitations (n = 3) and pleural effusion (n = 1), which were bilateral in 6 cases. CT scans of the head and brain magnetic resonance imaging (MRI) were consistent with the presence of brain abscesses in all the patients with central nervous system involvement.

The mean time between specimen collection and detection of the genus *Nocardia* was 27 days (SD, 12 days) by mycobacterial culture, whereas the mean time between the detection of the genus and the identification of species was 14 (SD, 8). Isolates were identified as *N. abscessus* (n = 5, 35.71%), *N. cyriacigeorgica* (n = 4, 28.57%) and *N. farcinica* (n = 4, 28.57%); the identification of *Nocardia* spp. was not available in one case only. *Nocardia* spp. were recovered from 6 different biological specimens: bronchial aspirate (n = 6), brain biopsy (n = 3), sputum (n = 2), subcutaneous abscess (n = 2), blood (n = 1) and peritoneal fluid (n = 1). *In vitro*, trimethoprim/sulfamethoxazole (TMP-SMX) and linezolid were uniformly active against all the isolates, whereas 40% of *N. abscessus* and 25% of *N. farcinica* showed resistance to carbapenems. Detailed information on susceptibility is summarized in *Table 2* and *Table 3*.

Overall, 12 patients out of 14 (85.71%) received an antimicrobial therapy. Immediately after the detection of the genus *Nocardia*, most patients (n = 10) received the genus-driven target therapy, an intravenous double-regimen antibiotic treatment which included, in the majority of cases, carbapenem (n = 8), TMP-SMX (n = 7), linezolid (n = 4) and ceftriaxone (n = 1), whereas a single-regimen with TMP-SMX was administered only in two cases.

All patients (n = 12) were switched to the species-driven target therapy as soon as the specie typing and the antibiotic susceptibility testing were available. This intravenous double-regimen antibiotic therapy, which lasted 4 weeks in all cases, included carbapenem (n = 7), TMP-SMX (n = 6), ceftriaxone (n = 4), linezolid (n = 3), levofloxacin (n = 1), ampicillin/sulbactam (n = 2) and amikacin (n = 1); in addition to intravenous treatment, one patient received amikacin by intrathecal route. In comparison to the genus-driven target-therapy, two regimens were optimized by switching from carbapenem to ceftriaxone, whereas other five regimens were

Table 2 - *Minimal Inhibitory Concentrations (MIC) of 14 strains of Nocardia spp. causing disease*

Patient	Nocardia *spp.*	*TMP-SMX*	*MEM*	*LZD*	*AMK*	*GEN*	*CRO*	*AMC*	*ERY*	*LVX*	*CIP*	*RIF*	*VAN*
1	*N. cyriacigeorgica*	ND	1	ND	0.75	ND	ND	8	ND	ND	4	ND	ND
2	*N. abscessus*	0.016	0.25	0.19	0.38	ND	0.5	ND	ND	ND	ND	ND	ND
3	*N. abscessus*	0.25	0.032	ND	32	ND	0.19	ND	ND	ND	ND	ND	ND
4	*N. farcinica*	0.047	1	0.25	0.75	ND	4	ND	ND	ND	0.5	ND	ND
5	*N. farcinica*	2	4	1	ND	ND	32	ND	ND	ND	1.5	ND	ND
6	*N. farcinica*	0.003	2	2	1	ND	3	0.25	ND	ND	2	ND	ND
7	*N. abscessus*	0.064	0.5	ND	ND	0.5	ND	3	48	2	ND	ND	16
8	*N. farcinica*	0.032	ND	0.064	ND	ND	2	0.25	ND	ND	ND	ND	ND
9	*N. cyriacigeorgica*	0.25	0.25	1	0.064	ND	1	ND	ND	ND	ND	ND	ND
10	*N. cyriacigeorgica*	0.047	0.047	0.094	0.047	ND	32	ND	ND	ND	0.5	ND	ND
11	*N. cyriacigeorgica*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
12	*N. abscessus*	0.75	1.5	0.25	0.5	ND	2.5	ND	ND	ND	32	ND	ND
13	*N. cyriacigeorgica*	ND	1.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
14	*N. abscessus*	0.002	1	0.19	0.125	ND	0.19	ND	ND	ND	2	ND	ND

Abbreviations: ND, not detected; TMP-SMX, trimethoprim/sulfamethoxazole; MEM, meropenem; LZD, linezolid; AMK, amikacin; GEN, gentamicin; CRO, ceftriaxone; AMC, amoxicillin-clavulanic acid; ERY, erythromycin; LVX levofloxacin; CIP, ciprofloxacin; RIF, rifampin; VAN, vancomycin.

Table 3 - *Identification by 16S rRNA PCR and percentages of susceptibilities of 13 strains of Nocardia causing disease.*

Antibiotic molecules	*N. abscessus (N = 5)*	*N. farcinica (N = 4)*	*N. cyriacigeorgica (N = 4)*
Trimetoprim/sulfametossazolo	100%	100%	100%
Imipenem	60%	75%	100%
Linezolid	100%	100%	100%
Amikacina	60%	75%	100%
Ceftriaxone	80%	0%	75%
Amoxicillina/clavulanato	40%	50%	50%
Eritromicina	0%	25%	25%
Levofloxacina	20%	75%	25%

modified due to the onset of adverse events. Specifically, three regimens were switched from TMP-SMX to linezolid, levofloxacin and ampicillin/sulbactam, respectively, because of the occurrence of hyperkalemia and two regimens were switched from linezolid to amikacin and ceftriaxone, respectively, because of the onset of thrombocytopenia. Once clinical status had improved, treatments were switched to oral single-regimen antibiotics (maintenance therapy), which included TMP-SMX (n = 7), amoxicillin/clavulanate (n = 3), minocycline (n = 1) and levofloxacin (n = 1).

Overall, the mean and median duration of antimicrobial therapy were 263.5 days (SD, 105.54 days) and 198.5 days (IQR, 178 - 352 days), respectively. Specifically, for localized pleuropulmonary involvement (n = 6), the mean and median duration were 192.67 days (SD, 29.94 days) and 188 days (IQR, 171 - 215 days). In the other involved sites, duration of antibiotic treatment were the following: 122 days in the primary soft tissue infection; 390 and 630 days, respectively, in the two disseminated diseases; 304 and 351 days, respectively, in the two brain abscesses; 315 days in the peritoneal nocardiosis.

One third (n = 4) of the treated population underwent one or more of the following surgical procedures: brain abscess aspiration (n = 3), implantation of Ommaya reservoir for both cerebrospinal fluid evacuation and intrathecal amikacin delivery (n = 1) and incision, drainage and debridement of subcutaneous abscesses (n = 2).

Overall, survival rate at one-year after completion of treatment was 100%; nevertheless radiological residual lesions were persistently detected by chest X-rays (n = 4) and brain MRI (n = 1) in five individuals after the discontinuation of treatment.

DISCUSSION

Considering the ubiquity of *Nocardia* spp., the isolation of these microorganisms from sputum or a skin swab is not always suggestive of invasive infection, as they may reveal laboratory contamination or colonization of the upper respiratory tract or skin. For this reason, even though *Nocardia* spp. was isolated in 26 subjects, only 14 were identified as infected and therefore enrolled in the study.

Considering that only one patient was not affected by an underlying diseases causing immunodepression, the comorbidity analysis of the study population confirmed the opportunistic nature of nocardiosis (Wilson *et al.,* 2012) (Valerio Minero *et al.,* 2009). As widely described in literature, the administration of corticosteroids has been the most commonly encountered risk factor in our series (Coussement *et al.,* 2016) (Peleg *et al.,* 2007) (Chouciño *et al.,* 1996).

Regarding the site of involvement, the pulmonary apparatus was the most common, followed by the central nervous system and the disseminated infection: these data are consistent with those presented in the review written by McNeil and Brown (1994), which included more than 1000 patients (McNeil *et al.,* 1994). The pulmonary lesions observed on the chest CT scan proved to be pleomorphic and not specific, as they were generally reported in literature by irregular nodules, cavitations and localized infiltrates (Sato *et al.,* 2016).

Lymphocyte typing at diagnosis was carried out on five patients only, due to the retrospective nature of the study. The mean and median CD4+ lymphocyte count was slightly below the reference range, suggesting that even a moderate reduction in their count could significantly increase the risk of developing nocardiosis.

Considering the collection sites of biological samples submitted for microbiological investigation, we observed that most of the *Nocardia* spp. were identified from specimens collected by invasive techniques (bronchial aspirates and brain biopsies), suggesting that these samples are more suitable for species identification.

Data available in literature confirm that *Nocardia* spp. infection provides positive results for serum BG, which is a cell wall component of many fungal species. Due to the retrospective nature of this study, serum BG was available at diagnosis only in 50% of the enrolled patients. Serum BG reached values higher than 400 pg/mL in most of the tested samples, thus proving to be a surrogate, yet non-specific, marker of nocardiosis. However, considering that we collected limited data on its potential confounding factors (i.e.: treatment with albumin or immunoglobulin, hemodialysis, sponges or gauze exposure, etc.) and that two cases of disseminated nocardiosis displayed a negative serum BG assay, these findings are inconclusive.

N. abscessus, originally *N. asteroides complex*, was responsible for one third of nocardiosis, differing significantly from the results reported by other epidemiological investigations, which displayed rates under 10%, and highlighting the key role of molecular identification tests (Uhde *et al.,* 2010) (Kageyama *et al.,* 2004). Indeed, a precise identification of species feeds back into antibiotic sensitivity patterns and, as a result, into the choice of antibiotic treatment: what was formerly referred to as *N. asteroides* was later found to be a group of species with a heterogeneous pattern of antibiotic susceptibilities. Noteworthy, 40% of isolates of *N. abscessus* showed resistance to carbapenems, which are usually recommended as an alternative for empirical therapy (Farran *et al.,* 2016) (Brown-Elliott *et al.,* 2016) (Wiseman *et al.,* 1995) (Velasco *et al.,* 1996).

The antibiograms of the strains isolated in this case series are substantially consistent with the results of the antibiotic susceptibility profiles available in literature (Schlaberg *et al.,* 2014) (Lebeaux *et al.,* 2014). Our data confirm that TMP-SMX and linezolid have the highest activity *in vitro*, regardless the isolated Nocardia spp.

The choice of antibiotics administered in the time period between the identification of the genus and the availability of antibiograms proved to be adequate in all cases. Despite its off-label prescription in the treatment of nocardiosis, linezolid was administered to one third of patients, demonstrating an optimal clinical efficacy.

An unconventional, wait-and-see approach was chosen in two cases, whose paucisymptomatic and localized pulmonary nocardiosis (both caused by *N. cyriacigeorgica*) remained stable at one-year follow-up, possibly representing an alternative approach in extremely selected cases, when the paramount aim is minimizing adverse events and drug interactions.

Despite the retrospective nature of this study and its small sample size, our findings add knowledge on the role of molecular tests in identifying the *Nocardia* spp., a key element for the antimicrobial stewardship. Further prospective studies are needed to assess both the role of serum BG in the diagnosis of nocardiosis and the possible threshold value of CD4+ lymphocyte count, in order to identify the subjects who may benefit the most from a prophylaxis with TMP-SMX.

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