Cutaneous Nocardiosis

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Abstract

Nocardiosis is a rare infection, potentially severe when the diagnosis is delayed and/or the response to treatment is ineffective because of multidrug resistance. The number of patients suffering from nocardiosis is constantly rising worldwide, and especially the occurrence among immunocompetent patients is a matter of concern. Surgical site infections, although rare, should be considered among risks factors. Skin manifestations are often the first signs of the infection, both for direct inoculum (primary cutaneous infection) or disseminated disease. Clinicians might be misguided from the variable, unspecific, polymorphous skin eruptions, simulating more common diseases. Especially in the immune suppressed patients symptoms might evoke the same disease for which the patient is under treatment. The dermatologist's expertise and high level of suspicion are crucial to settle the extensive differential diagnosis, cooperating with the pathologist, and microbiologist to find out the causative organism. Molecular methods of identification could improve diagnosis and the chances of survival, but are not routinely performed in many laboratories.

Keywords: *Nocardia* infection; Primary cutaneous nocardiosis; Disseminated nocardiosis; Risk factors; Differential diagnosis

Cutaneous Nocardiosis

Nocardiosis is a rare human infection reported worldwide [1-7] due to ubiquitous soil born gram-positive, filamentous aerobic bacteria, initially confused with fungi although their branching filaments are thinner than true hyphae (1-2 μ m vs 3-5 μ m in diameter). *Nocardia* genus shows similarities with other filamentous bacteria (nocardioforms), therefore grouped under the Actinomycetales order [8]. The disease is more frequent in tropical countries, without age or ethnic predilection, although slightly more common in males than females [1,2]. Male predominance is probably related to the type of working and environmental exposure to the infection, rather than to genera differences [9]. In western countries the prevalence is probably underestimated [4,7,9] and last decade increased reporting has changed the common attitude to consider it just an opportunistic infection, with approximately 1/3 of immunocompetent patients affected, and a considerable risk associated with major surgical procedures or trauma [1-9].

First signs are frequently cutaneous, either as part of a systemic infection disseminated to the skin, or as a primary

cutaneous inoculation. Primary cutaneous nocardiosis is more common, with incidence rates ranging from 5% to 24% in various countries like India, UK, and Spain [10-12]. A major problem is correct identification, as clinical presentation is not peculiar as well as histological findings, suggestive of an infective process, but not able to detect the microorganisms, unless a tissue culture has been performed and Gram stained. The laboratory should be advised of the clinical suspicion, as *Nocardia* is a slow-growing bacterium, and cultures are often discarded when no growth is seen within 48 hours, or obscured by rapidly growing bacteria [1].

Once the Nocardiosis is presumed, speciation might take 1-3 weeks, and each strain has individual antibiotic susceptibility pattern, conditioning empiric treatment and possible fatal course. Newer molecular technologies promise to provide rapid and accurate identification [13], but first of all have revolutionized taxonomy, and we have to face with currently more than 30 species. Clinical relevance has been determined for about seventeen species [14,15] in respect to the 4 pathogens identified by traditional hydrolysis patterns: *N. asteroides*, *N. brasiliensis*, *N. otitidiscaviarum*, and *N. transvalensis*. Distinct serotypes and biotypes might share the same antibiotic susceptibility profile [8,13-18]. In western Europe and USA, the term "*N.asteroides* complex" is frequently used, failing to differentiate the strain cluster (*N.asteroides sensu strictu*) from *N. cyriacigeorgica*, *N. farcinica*, *N. nova*, *N. abscessus*, *Nocardia brasiliensis*, which is the second most common isolates in the majority of countries [14,16], differs from *N.pseudobrasiliensis* in mycolic acid pattern, adenine decomposition, nitrate reduction, and antimicrobial agent susceptibilities [18] For all these issues, clinical management and treatment of patients with nocardial disease remain a challenge. This review characterizes the cutaneous manifestations and related risk factors, to suggest clues in clinical diagnosis, raise physicians alert to avoid delayed recognition and address the correct assessments.

Skin manifestations

From a general health perspective, cutaneous nocardiosis (CN) traditionally occurs with 3 main patterns: (1) actinomycetoma, (2) lymphocutaneous (sporotrichoid) infection, (3) superficial skin infection (Table 1). Some classification adds a distinct 4th pattern, corresponding to the secondary skin

Table 1: Cutaneous nocardiosis: clinical patterns, responsible strains and differential diagnosis.

	Main species	Differential Diagnosis	Course	Empirical therapy
Mycetoma	<i>N. brasiliensis</i> <i>N. pseudobrasiliensis</i>	Eumycetoma Botriomycetoma Skin malignancies	Chronic	TMP/SMX + Minocycline
Lymphocutaneous infection	<i>N. brasiliensis</i> <i>N. pseudobrasiliensis</i> <i>N. abscessus</i> <i>N. asteroides</i> <i>N. farcinica</i> <i>N. nova</i>	Sporotrichosis Atypical mycobacteriosis Other deep fungal infections	Subacute	TMP/SMX Minocycline; Amoxicillin-clavulanic acid, linezolid, ceftriaxone
Superficial skin infection / Secondary dissemination	<i>N. asteroides</i> <i>N. abscessus</i> <i>N. farcinica</i> <i>N. nova</i> <i>N. asiatica</i> <i>N. transvalensis</i> <i>N. otitidiscaviarum</i>	<ul style="list-style-type: none"> Other bacterial Infections and bacteremic lesions Inflammatory conditions: Erythema nodosum; pyoderma gangrenosum; Sweet syndrome; other vasculitis Skin Metastasis 	Acute or subacute	Very resistant strains: Amikacin + Imipenem/ meropenem, Linezolid

infection, due to dissemination from a pulmonary disease [19]. Besides, there are not unique distinctive elements allowing the clinical differentiation of a primary superficial skin infection from a secondary dissemination. From our experience, the severity, number or distribution of the lesions are not directly evocative of the general state of the patient, as internal organs infection might present with isolate quite indolent subcutaneous nodules (Figure 1), while by converse, very severe diffuse lesions, accompanied with fever and general malaise in other patients were not due to an hematogenous spread (Figure 2). Dissemination to the skin is estimated to occur in approximately 20% of patients with systemic nocardial infection [3]. Chronic or underestimated skin infection might be at any moment complicated by dissemination to the internal organs [1,8,19] although an estimation of such risk is not currently reported. Thus, any skin manifestation suspect for nocardial infection requires a complete assessment for internal organs involvement.

Actinomycetoma is also called Madura Foot after the Indian region where the disease was firstly described in 1842 [20]. It is the most frequent presentation in tropical countries, especially Mexico, and South America [1,8,21], but it is also sporadically reported in temperate countries [22-24]. The incubation period varies from several weeks to months. Other than the lower extremities, the hands and forearms might be affected. *Nocardia* mycetoma is clinically indistinguishable from the Eumycetoma type, which is due to fungi, and the Botriomycetoma type, which is due to other bacteria (*S. aureus*, *Pseudomonas Aeruginosa*, *E.coli*, *Proteus mirabilis*, etc.). Initial lesions might be characterized by quite indolent erythematous tumor-like soft-tissue swelling, in which multiple subcutaneous nodules are palpable, progressively tending to ulcerate, and draining a peculiar purulent material, with pale yellow granules (sulfur grains). The deep infection has a chronic course, with granulomatous and fibrotizing evolution, leading to multiple sinus tracts formation, covered with hypertrophic verrucous skin (Figure 3) and later progressive destruction of subcutaneous tissues, fascia, and muscles, causing deformation and functional impairment. Osteomyelitis is a frequent complication, and plain

x-ray assessment or CT scan is advisable to depict bony osteolytic areas (moth eaten) and eventual periosteal reaction. The “dot-in-circle” sign has recently been proposed as a highly specific early sign of mycetoma on magnetic resonance imaging (MRI) [25,26], consisting of multiple small cystic subcutaneous areas with central hypointensity dots. The sign has been applied also to ultrasonography (USG), -where the circle areas are hypoechoic with central hyperechoic foci [27]. Morbidity is significant, with persistence over years, and even incurable cases, leading to wide ablative surgical intervention or final amputation [28].

Lymphocutaneous nocardiosis is the most unusual form [29,30] or probably underestimated for the similarity with sporotrichosis, a more common deep fungal infection characteristically spreading along lymphatics after a skin injury. A certain predilection for children has been noted [31,32]. Lower

Figure 1: A painless erythematous swollen patch with few hemorrhagic vesicles and crusting on the left elbow of a 65 year-old white man, under high doses corticosteroid therapy for a recent diagnosis of *Pemphigus vulgaris*. Laboratory culture from the skin specimens allowed identification of *Nocardia* colonies, further identified by PCR as *N. asteroides*. Radiologic imaging assessed the presence of a pulmonary and brain involvement.

extremities are more affected, but hands follow, especially in patients with a history of gardening or thorn injuries, preceding of several days the eruption onset (Figure 4). Sometimes the port of entry is hardly detectable, otherwise affected with a swollen erythematous excoriation or ulcer, but the attention is attracted from the multiple erythematous painful nodules or satellite pustules linearly distributed on the main veins and lymphatics, proximally moving with lymphangitis striae towards the regional lymph nodes. The course is subacute, usually requiring long-term antibiotic therapy (2-6 months), and possible relapses.

Superficial skin infection is a very polymorphous pattern, wide spread erythematous papules and pustules (Figure 2 & 5), sometimes bullae, mixed with subcutaneous nodules similar to erythema nodosum, but less painful. All these elements (multiple mycetomas) might produce abscesses and ulcers, discharging variable content of pus, necrotic and hemorrhagic materials (Figure 2). Multiple lesions might appear on an erythematous edematous plaque, with ill-defined borders resembling cellulitis

Figure 2: A severe cellulitis of the left leg, extending on the foot and ankle, with erythematous haemorrhagic papules and pustules draining pus and sero-haematic material, subcutaneous nodules, necrotic ulcers. The 58 year-old white man was otherwise healthy, with a history of minor trauma while gathering wood 2 weeks before the eruption onset. Cultures from the skin specimens allowed identification of *Nocardia* colonies, further identified by PCR as *N. brasiliensis*. Any systemic involvement was excluded.

Figure 3: Madura foot in a 65 year-old white man, working as farmer with a history of venous insufficiency and chronic lymphoedema, but not reporting a specific trauma history. Although susceptible to conventional antibiotic therapy, the coalescent hypertrophic nodules of the ankle required repeated surgical interventions.

Figure 4: A lymphocutaneous nocardiosis in a 26 year-old white healthy gardener, with a history of rose thorn injury several weeks before the eruption onset. Tissue cultures from a skin biopsy suggested the *Nocardia* responsibilty, and further laboratory identification isolates *N. asteroides*.

Figure 5: Superficial skin nocardiosis of the right leg and knee in a 66 year-old white man, under low doses corticosteroids for chronic rheumatoid arthritis. A minimal port of entry was noted (yellow arrow) where the patient referred an insect bite. The central hospital laboratory identified *N. asteroides* from the skin cultures, but optimal response to amoxicillin-clavulanic acid might suggest *N. abscessus* responsibility. Molecular methods were not available at that time.

[33]. Extremities are most commonly affected, and unilateral distribution might be suspicious of a primary cutaneous exposure, but solitary lesions on different body sites are also reported in cases in which a systemic disease has been accurately excluded [34]. Manifestations mimic more common infection, such as *Streptococcus* or *Staphylococcus* species, and antibiotic empirical treatment might be efficacious in most of the cases. Cultures are not always performed in daily practice, because time and money consuming, until the patient worsens, and complete assessment are performed. A study realized in Texan children documented the occurrence of *Nocardia brasiliensis* in cellulites or abscess cases

not improving with appropriate antibiotics [32]. Another crucial point is differential diagnosis from not infective conditions, such as erythema nodosum, pyoderma gangrenosum, and other type of vasculitis, especially in patients under immunosuppressive treatment for autoimmune diseases, such as lupus erythematosus or Crohn's disease, primarily evoking or associated with similar skin manifestations [33-37]. Subcutaneous nodules occurrence have been suspected for pulmonary cancer metastasis in a patient with chronic myelogenous leukaemia [38].

The course of superficial skin nocardiosis is variable, being considered the least serious form of nocardiosis [13], although worsening or relapsing despite adequate management is common. A fatal outcome occurs exclusively in disseminated nocardiosis, especially when *N. farcinica* is implicated [2,5,38,39].

Source of infection and risks factors

Nocardial natural habitat is the soil, as part of the normal microflora, and therefore the different species with geographical variations might be present in dust, sand, decomposing plant material, but also associated with water, including swimming pool [8]. Especially warm windy conditions might facilitate air dispersal of the microorganisms. Animals such cattle and dogs might be affected [40], but transmission to humans is unusual, as well as inter-human infection. Nosocomial outbreaks have been reported, most frequently in immunocompromised, but also immunocompetent patients intra-operatively exposed to *N. farcinica*. Contamination of arterial stent, prosthesis, or heart/liver transplantation units transmission via health care workers has been documented, posing the question of intensified hand-washing regimen, correct barriers use (gloves, gowns), and environment cleaning [41,42]. Some species are more associated with distinct type of human infections, thus the modality of infection and risk factors might be different, considering if the skin has been directly contaminated or by the blood stream dissemination. Several Authors report that primary cutaneous nocardiosis is more common than disseminated infection [10,19,21], but large general hospital studies are usually dedicated to systemic nocardiosis, in which skin involvement is only partially considered [2-5].

Direct inoculation to the skin (Primary cutaneous nocardiosis) involves both compromised hosts and immunocompetent individuals, with a history of trauma. It is most commonly caused by *Nocardia brasiliensis* [10,21,29,43,44], but any of the pathogenetic nocardiae are able to infect the skin, including recently described entities, such as *N. nova* and *N. asiatica* [16,17,45,46]. More aggressive cases are related to *N. farcinica* and *N. pseudobrasiliensis* infections [1,18,28,34]. Considering their ubiquitous distribution, it is plausible that the *Nocardia* infection has a self-limited course in the majority of cases. Neutrophils are early involved during infection, limiting the spread, but are not able to kill the *Nocardia*, whose filaments inhibit phagosome-lysosome fusion, and resist the oxidative killing mechanism of phagocytes. Thus, activation of macrophage and cytotoxic T lymphocyte intervention is necessary to effectively clear the intracellular pathogens, while humoral

immunity has a less defined role. The host-parasite relationship and virulence determinants have been magisterially described by Beaman and Beaman [9].

Considering the necessity of a port of entry, main risks factors are:

- i. Puncture or other contaminated traumatic inoculum, such as thorn, insect or animal bite, scratch, bullet injury, intravenous drug abuse [9,19,47,48]. Occupation like farmer, shepherd, gardener, and an history of rural background or barefoot walking may help in typical pedal mycetoma, but unusual site are also to be considered, such as the face, neck, breast, back or even the scalp, after a car incident, and the vulva simulating an advanced carcinoma [31,49-52].
- ii. Diabetes, malnutrition, alcohol abuse are predisposing conditions, as in any kind of infections.
- iii. Several studies have documented that steroids administration is the main independent risk factor for development of *Nocardia* infection [2,53]. Corticosteroid use associated with nocardial disease has been reported in several autoimmune disease and inflammatory conditions (lupus erythematosus, Crohn's disease, autoimmune blistering diseases, sarcoidosis, etc.) [35-37,54-57].
- iv. Infection at site of surgery is increasingly reported, from biopsy to major procedures, such as open heart sternotomy, vascular prosthesis and central venous catheters insertion [41,42,58,59]. Severe postoperative wound infections are conditioned by the virulence of the strain, but also affinity to medical material, and resistance to common antibiotic prophylactic regimen. Further dissemination is common, but appears to have a more benign course in respect to the dissemination phase of a systemic nocardiosis [13].

Blood stream dissemination to the skin usually results from pulmonary disease, caused by direct inhalation of contaminated particles from soil or water, and development of a rather asymptomatic focal pneumonitis, frequently undervalued until secondary symptoms occurrence [1]. *Nocardia bacteriemia* is rare, although related to a high mortality rate [39,53,60]. The microorganisms seem to have a predilection for the lung and central nervous system (CSN) involvement [61], followed by the skin tropism, which is estimated to occur in approximately 10-20% of patients [1,2]. Disseminated nocardiosis typically affects immunocompromised patients, with underlying HIV infection, malignancies, chronic lung disease, solid-organ transplants or previous long-term use of corticosteroids, configuring an opportunistic infection [3,15,53,62,63]. Incidence in these high risk patients is 140-340 folds higher than in the general population [64]. Less than 10% of patients with disseminated nocardiosis have no identified underlying predisposing factors [15]. The main isolated strain worldwide is *Nocardia asteroides*, which is part of an heterogeneous taxon following the new

classification [1,13]. Major case collection in Italy have been studied by a hospitals collaborative network of 11 cities [7], confirming prevalence of *Nocardia asteroides sensu strictu*, followed by *N. farcinica*, *N. nova*, and *N. brasiliensis*. By converse, in Belgium, and Germany the *N. farcinica* isolate has emerged as the predominant pathogen [65,66], and a review of reported Asian, European, Nord American cases suggest that this isolate occur more frequently than previously recognized [67], showing a more virulent behavior and resistance to several antibiotics (cefotaxime, cefamandole, tobramycin). Another 17- 20% of isolates under the *N. asteroides* complex is due to *N. nova*, firstly characterized by Tsukamura, and responsible of the same spectrum of clinical manifestations, but unique susceptibility to ampicillin and erythromycin [67,68].

Diagnosis

Nocardiosis is a complex diagnosis, because conventional microscopy and culture have current limitations, and molecular methods are not routinely disposable in daily practice. Dermatologist's high index of suspicion is mandatory to alert the pathologist, microbiologist and other clinicians involved in the assessment. Several specimens should be taken from the skin lesions, because often cultures result negative, although a single nocardial colony is highly indicative, being not usual laboratory contaminants. Skin biopsy for histopathology is necessary to exclude other inflammatory conditions and orient towards an infective process, characterized by a mix middle and deep dermis infiltrate, with abundant neutrophils, nuclear dust and abscesses formation. Focal extension into the subcutaneous fat is frequent. Old mycetoma lesions might be more specific, with palisading histiocytes surrounding the neutrophilic infiltrate and the characteristic grains. Several special stains are used to complete conventional hematoxylin and eosin (H&E) observation without usually detecting the microorganisms (Gram; Periodic acid-Schiff (PAS); Gomori methenamine silver (GMS); Ziehl-Nielsen). The most reliable and sensitive methods of diagnosis remains the tissue culture execution, stained with Gram, showing the Gram-positive branching rods [15]. A fast-acid positivity is detectable for the majority of the *Nocardia* species, but the fuchsin bound is fable and peculiar weak acid decoloration is suggested (modified Kinyoun stain). The property is transient, generally lost in sub-cultured colonies [1].

Radiological imaging is necessary to exclude an eventual systemic nocardiosis, and to assess the extent of subcutaneous tissues involvement, although conventional radiography is diagnostic in the advanced stages. Clues to early diagnosis (dot-in-circle sign) have been suggested for ultrasound echography and magnetic resonance imaging [25-27]. Routine blood cultures are usually negative, while blood haemato-chemistry exams confirm the inflammatory state, with frequent white cells count increase, prominently neutrophilis, serum C-reactive protein, erythrocyte sedimentation rate, and serum protein electrophoresis anomalies. No serodiagnosis is currently available to identify active nocardiosis, nor skin tests to demonstrate delayed cutaneous hypersensitivity [1,9].

Laboratory identification is the final conclusive diagnosis, but is difficult in routine practice. Presumptive identification from cultures follows a long sequence of steps, from microscopic phenotypic morphology, to lysozyme resistance, hydrolysis patterns (casein, tyrosine, xanthine, hypoxanthine), biochemical reactions to several sugars and other carbon substrates; arylsulfatase use; growth patterns at 35°C and 45°C. Practical indications, algorithm and flowcharts have been suggested for laboratory consultation [15,65], but the time frame required to answer clinician's expectations varies from a minimum of 7 days to several weeks. The most rapid and actual recommended tools for *Nocardia* species identification are the 16S rRNA gene sequencing and PCR (polymerase chain reaction) restriction fragment length polymorphism (RLFP) [13], but few research laboratories dispose of such procedures. The very crucial point is to obtain a reliable antibiotic susceptibility test to guide the treatment adjustment. Predictive susceptibility has been identified for the main pathogen species, with a VI type classification on the base of the drug patterns [13,17,65]. Unfortunately, antimicrobials susceptibility tests (AST) for *Nocardia* species are difficult to realize in common laboratories, and not conclusive standardized, validated procedures have been agreed, especially considering the recently demonstrated heterogeneity of the genus and interregional variability of the isolates [1,5,8]. Guidelines for best laboratory practice have been provided [69] but correlation with clinical outcomes has not been systematically performed.

Therapy

All antimicrobial categories have been used in *Nocardia* infections, but "gold standard" treatment has not been achieved. The association of erythromycin+ampicillin or sulfonamide+ampicillin was among the most used in past decades, but several aggressive strains are resistant to ampicillin. *N. farcinica*, *N. cyriacigeorgica*, *N. brasiliensis* and *Pseudobrasiliensis*, *N. otitidiscaviarum* [13]. Sulfa-containing agents remain the drug of choice, in combination with trimethoprim [1], variously associated with minocycline, amoxicillin-clavulanate, 3rd generation cephalosporins, amikacin, imipenem, fluoroquinolones, linezolid. Ideally, drug selection should be guided from individual antimicrobials susceptibility tests (AST), but laboratory results have long frame time, and empiric therapy is usually administered. The cutaneous pattern of nocardial infections is therefore useful to address the choice (Table 1), following the drug pattern classification [68].

Mycetoma, and lymphocutaneous nocardiosis, which are mainly due to *N. brasiliensis*, are most frequently treated with the association trimethoprim+sulfamethoxazole (TMP-SMZ), because of historical high susceptibility and clinical efficacy, sometimes despite the in vitro indication of strain resistance [1,47]. Most of the *N. asteroides* complex isolates are also susceptible, as well as *N. transvalensis*, while *N. otitidiscaviarum* is resistant both in vitro and in vivo, but is rarely implicated [8,13,15,17]. Beside, severely immunocompromised patients for organ transplants or HIV-infection are frequently intolerant to such therapy, developing skin rash to severe hypersensitivity reaction, persistent myelosuppression and increased concomitant drugs toxicity [1,8]. Minocycline has excellent in vitro activity, and is usually

associated with other bactericidal agents, especially TMP-SMZ, for the additional anti-inflammatory activity [70].

Treatment duration is another crucial point, usually extended for several months after complete clinical recover, because of the high numbers of relapses [15]. Antimicrobial resistance might occur in course of initially efficacious treatments, with sudden worsening. Repeated susceptibility testing should be considered, especially in severe or refractory cases.

Superficial skin infection, as well as mycetoma severe or refractory cases might require wide spectrum antibiotics, also considering the possibility that lesions are part of a disseminated nocardiosis, before all assessment have been performed. Animal models and in vitro data attest imipenem and amikacin superior activity compared with TMP-SMZ and also synergistic effects combining the 3 drugs, but parenteral administration requires hospital settings or expensive out-patient facilities, not affordable for long-term treatment. Limited activity in vitro is suggested for fluoroquinolones, although clinical experiences found it successful in long-term combinations regimens [8,42]. Thus, clinician's decisions should be guided by the single patient conditions:

- i. Mild to moderate infections should initiate with oral TMP-SMZ alone or in association with minocycline. When sulfonamides are contra-indicated, ampicillin-clavulanic or fluoroquinolone preparations can be administered. If the clinical response is slow, a parenteral therapy should be considered, but usually at that time individual antimicrobial susceptibility results are ready to consultation.
- ii. Very severe patients require parenteral therapy from the very beginning, with a combination of imipenem/meropenem; amikacin; cephalosporins or linezolid for 3-6 weeks, than switching to oral preparations if improvement or on the basis of AST results.
- iii. Treatment failure can occur for sequestered abscesses and persistent fibrotizing soft tissues inflammation, demarcating the mycetomas, and requiring additional drainage or surgical procedures [28]. Secondary or coexistent infections might complicate the course of skin lesions, especially in the compromised hosts [11].

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