Molecular identification and susceptibility pattern of clinical *Nocardia* species: Emergence of *Nocardia crassostreae* as an agent of invasive nocardiosis

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**BACKGROUND:** Nocardia species are rare, opportunistic organisms that cause disease in both immunocompetent and immunocompromised individuals.

**OBJECTIVE:** To investigate the clinical presentations of various Nocardia infections based on the 16S ribosomal RNA gene of the isolate, as well as related risk factors and susceptibility patterns to antimicrobial agents.

**METHODS:** Thirteen patients with a diagnosis of nocardiosis were included in the present study. Seven Nocardia species were identified by 16S ribosomal RNA. Susceptibility testing was performed using six antimicrobial agents.

**RESULTS:** Five patients were immunocompromised, and eight were immunocompetent with predisposing factors including cystic fibrosis, tuberculous and ophthalmic infections. Nocardia caused pulmonary infections in eight patients (61.5%), invasive systemic infections in three patients (23%) and local (ophthalmic) infections in two patients (15.4%). In the patients with pulmonary disease, nocardiosis was caused by six species (*Nocardia cyriacigeorgica, Nocardia otitidiscaviarum, Nocardia farcinica, Nocardia carnea, Nocardia testacea and Nocardia asiatica*). The seventh species identified in the present study was *Nocardia crassostreae*.

**DISCUSSION:** *Nocardia crassostreae* is a multidrug-resistant organism that was reported to be an emerging human pathogen causing invasive nocardiosis in a patient with non-Hodgkin’s lymphoma. *N farcinica* was isolated from blood in a patient with breast cancer. None of the Nocardia isolates were resistant to linezolid. One *N otitidiscaviarum* isolate was a multidrug-resistant organism. All patients in the present study were treated with the appropriate antibiotics and their condition resolved without further sequelae.

**CONCLUSIONS:** The present study is the first report on *N crassostreae* as a human pathogen. The detection of multidrug-resistant species necessitate molecular identification and susceptibility testing, and should be performed for all *Nocardia* infections. Nocardiosis manifests various clinical features depending on the *Nocardia* species and underlying conditions.

**Key Words:** Antimicrobial susceptibility; Clinical cases; Molecular identification; Nocardia; *Nocardia crassostreae*

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Nocardiaceae are ubiquitous organisms distributed worldwide in the environment as saprophytic components of fresh and salt water, soil, dust, decaying vegetation and decaying fecal deposits from animals (1). Nocardiaceae are rare opportunistic organisms that cause diseases in immunocompetent and immunocompromised individuals, and are reported in all ages and ethnic groups. Immunocompetent patients usually develop localized cutaneous lesions, such as cellulitis, abscesses or sporotrichoid forms (2), and endogenous endophthalmitis (3). However, most cases are reported in immunocompromised patients, and manifest as deep infections or disseminated diseases (4-6). Risk factors for infection with Nocardiaceae include solid organ transplant (5,7-9) or bone marrow transplant recipients (10), patients with hematological diseases (11,12) and HIV infection (13-16). Pulmonary nocardiosis can be a cause of disease in populations with risk factors such as immunompression, malignancies and severe lung disease (17), patients with cystic fibrosis (18), and in patients with chronic obstructive pulmonary disease and bronchiectasis (19,20). Lung infections are frequent and, in many cases, disease can spread to the central nervous system, including the brain (4,21), with poor prognosis in some cases, irrespective of antimicrobial therapy (22).

Identification of clinical isolates beyond the genus level is important because Nocardia species differ in clinical spectrum and their susceptibility to antibiotics. The classic laboratory methods of identification, including direct examination and culture, are insufficient; therefore, sequence analysis of the 16S rRNA gene is mainly used for the identification of Nocardia isolates to species level (23,24).

Trimethoprim-sulfamethoxazole has traditionally been the agent of choice for the treatment of nocardiosis, with alternative drugs including amikacin and imipenem (1,25). Resistance and therapeutic failure may occur, which necessitates a search for alternative agents. The aim of the present study was to investigate the clinical presentations of various Nocardia infections based on the 16S RNA gene of the isolate, related risk factors and susceptibility patterns to various antimicrobial agents.

METHODS

Patients

The present laboratory-based study was approved by the Scientific Council and Ethics Committee of Hamad Medical Research Center, Doha, Qatar (proposal number 10174/10). Thirteen patients of different nationalities with various clinical symptoms and risk factors admitted to Hamad Hospital, Doha, Qatar, were diagnosed with nocardial infections by the main Microbiology Laboratory of Hamad Medical Corporation from January 2006 to June 2010. Patients’ clinical records, demographic data and treatment outcomes were included in the present study. Information regarding sex, age, underlying conditions including history of immunosuppressant drug use, tuberculosis and malignancy were analyzed (Table 1). Disseminated nocardiosis was considered for infection of two organs or more, such as lungs, lymph nodes, brain or blood. The respiratory samples included sputum, endotracheal aspiration and bronchoalveolar lavage. A diagnosis of pulmonary nocardiosis required at least one positive culture from respiratory samples, and the presence of clinical symptoms and an abnormal chest radiograph.

Isolation and identification of Nocardia species

A total of 13 clinical specimens positive for Nocardia species were recorded over a four-year period. Nocardia species were isolated and identified according to standard laboratory procedures. Identification of Nocardia species was based on Gram-positive branching, beaded and filamentous bacilli, and positive modified acid-fast stain results. The clinical specimens were generally cultured on chocolate agar and blood agar media, and incubated in both aerobic and anaerobic conditions at 37°C. Characteristic dry, chalk-like Nocardia colonies appeared on aerobic cultures after three to seven days of incubation, depending on the species. Blood cultures were performed using the Bactec automated culturing system (BD Diagnostic Systems, USA).
Clinical and molecular characterization of Nocardiopsis

### TABLE 1
Clinical characteristics and outcome of 13 patients with nocardiosis

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/years</th>
<th>Patient origin</th>
<th>Clinical diagnosis</th>
<th>Clinical specimen</th>
<th>16S rRNA identification (genus Nocardiopsis)</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42/F</td>
<td>Qatar</td>
<td>Non-Hodgkin’s lymphoma, abscess on back at L1 level</td>
<td>Pus aspirate</td>
<td>N cyriacigeorgica</td>
<td>Ceftriaxone + SXT</td>
<td>Recovered</td>
</tr>
<tr>
<td>2</td>
<td>33/M</td>
<td>India</td>
<td>Chest pain, pleural effusion, lingular infiltrates</td>
<td>Sputum</td>
<td>N cyriacigeorgica</td>
<td>Azithromycin</td>
<td>Recovered</td>
</tr>
<tr>
<td>3</td>
<td>36/M</td>
<td>India</td>
<td>Pulmonary infection mimicking tuberculosis</td>
<td>Sputum</td>
<td>N cyriacigeorgica</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>45/M</td>
<td>India</td>
<td>Corneal abscess</td>
<td>Corneal scraping</td>
<td>N otitidiscaviarum</td>
<td>Local gentamicin and levofloxacin eye drops</td>
<td>Recovered</td>
</tr>
<tr>
<td>5</td>
<td>17/F</td>
<td>Qatar</td>
<td>Cystic fibrosis with pneumonia</td>
<td>BAL</td>
<td>N otitidiscaviarum</td>
<td>Clarithromycin + SXT, moxifloxacin</td>
<td>Recovered</td>
</tr>
<tr>
<td>6</td>
<td>50/M</td>
<td>Qatar</td>
<td>Conjunctivitis</td>
<td>Conjunctival swab</td>
<td>N farcinica</td>
<td>Fusidic acid + lomefloxacin</td>
<td>Recovered</td>
</tr>
<tr>
<td>7</td>
<td>68/M</td>
<td>Qatar</td>
<td>Renal transplant</td>
<td>Sputum</td>
<td>N farcinica</td>
<td>Meropenem + SXT</td>
<td>Recovered</td>
</tr>
<tr>
<td>8</td>
<td>50/F</td>
<td>India</td>
<td>Breast cancer on chemotherapy with central line-related sepsis</td>
<td>Blood</td>
<td>N farcinica</td>
<td>SXT</td>
<td>Recovered</td>
</tr>
<tr>
<td>9</td>
<td>30/M</td>
<td>India</td>
<td>Pulmonary tuberculosis, bronchiectasis</td>
<td>Sputum</td>
<td>N carnea</td>
<td>Anti-TB</td>
<td>Recovered</td>
</tr>
<tr>
<td>10</td>
<td>36/M</td>
<td>Egypt</td>
<td>Pulmonary infection mimicking tuberculosis</td>
<td>Sputum</td>
<td>N carnea</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>11</td>
<td>73/F</td>
<td>Qatar</td>
<td>Non-Hodgkin’s lymphoma, paravertebral abscess at L3-L5, on chemotherapy</td>
<td>CT-guided aspirated fluid</td>
<td>N crassostrea</td>
<td>Meropenem + SXT</td>
<td>Improved; died from progressive disease</td>
</tr>
<tr>
<td>12</td>
<td>34/M</td>
<td>India</td>
<td>Previous case of pulmonary tuberculosis</td>
<td>Sputum</td>
<td>N testacea</td>
<td>None</td>
<td>NA</td>
</tr>
<tr>
<td>13</td>
<td>44/F</td>
<td>Syria</td>
<td>Previous case of pulmonary tuberculosis</td>
<td>Sputum</td>
<td>N asiatica</td>
<td>SXT</td>
<td>Recovered</td>
</tr>
</tbody>
</table>

**Anti-TB** Antituberculosis treatment; **BAL** Bronchoalveolar lavage; **CT** Computed tomography; **F** Female; **M** Male; **NA** Not available; **rRNA** Ribosomal RNA; **SXT** Trimethoprim-sulfamethoxazole

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**Figure 1** Magnetic resonance image of a patient (case 1) with pulmonary nocardiosis and dissemination to an abscess approximately 3.5 cm in diameter with thick wall (arrow) in the left lumborum muscle at level of L1 vertebral body caused by Nocardiopsis cyriacigeorgica.

**Figure 2** Computed tomography scan shows a large abscess caused by Nocardiopsis crassostreae, in the left psoas muscle (solid arrow) extending outward and producing a multiloculated abscess (open arrow) in the left lumborum muscle (case 11).

Invasive infections:

Patients with non-Hodgkin’s lymphomas (cases 1 and 11): Case 1 was a female patient with a Nocardiopsis cavitary lesion in the left upper lung lobe who developed an abscess approximately 3.5 cm in diameter with thick wall present at the left lumborum muscle at the level of L1 vertebral body, as shown in magnetic resonance imaging (Figure 1). Under computed tomography (CT) guidance, a large-gauge needle was inserted into the abscess and a small amount of pus was aspirated and sent to the microbiology laboratory for microscopy and culture; the culture grew N cyriacigeorgi. The patient recovered after treatment with ceftriaxone and sulfamethoxazole-trimethoprim (cotrimoxazole) and is still doing well. In the other woman (case 11), a CT scan revealed a large, paravertebral abscess at the L3-L5 level, present in the left psoas muscle and compressing and displacing the left kidney. A catheter was inserted percutaneously into the abscess; more than 50 mL of pus was aspirated and the catheter was left in situ for further drainage. The abscess extended outward and produced another abscess collection in the left lumborum muscle (Figure 2); the abscess was aspirated under CT guidance, and sent to the microbiology laboratory for microscopy and culture (the culture grew N crassostreae). There was no patient history of contact with or ingestion of molluscs. The patient improved after treatment with meropenem and trimethoprim-sulfamethoxazole, but died later from progressive disease.

Blood stream infections: The third case of invasive nocardiosis was found in an immunocompromised patient with breast cancer undergoing...
chemotherapy (case 8), who was diagnosed with *N. farcinica* infection. She was successfully treated with trimethoprim-sulfamethoxazole.

**Pulmonary nocardiosis**

Pulmonary nocardiosis was diagnosed in eight patients in the present study. In case 3, an immunocompetent male patient with chest pain, pleural effusion and lingular infiltrates, a CT scan showed a small area of consolidation present in the inferior segment of lingula of the left lung, along with ill-defined hazy linear shadowing present at the periphery of the lateral segment of the left lower lobe (Figure 3). Sputum culture grew *N. cyriacigeorgica*, and the patient recovered after treatment with azithromycin.

**Antimicrobial susceptibility**

The antimicrobial susceptibility patterns of the 13 *Nocardia* species for six antibacterial agents are summarized in Table 2. Growth inhibition ellipses were uniform and well delineated, and the points of intersection with the Etest strips were easy to determine, except for *N. otitidiscaviarum* (case 4), in which the mutant colonies showed variability in the results of nitrocifin test, with one of the antimicrobial agents cefotaxime and imipenem. Eight of the 13 cases were susceptible to linezolid, but showed various susceptibility patterns to other antimicrobial agents. The *N. cassinum* isolate (case 11) was resistant to amikacin, cefotaxime and imipenem. The *N. otitidiscaviarum* isolate (case 4) was resistant to imipenem, cotrimoxazole and cefotaxime, and intermediate for moxifloxacin. *N. otitidiscaviarum* isolate (case 5) was resistant to the antimicrobial agents cefotaxime and imipenem. Eight *Nocardia* isolates showed beta-lactamase activity by nitrocefin disk test. Positive reaction was demonstrated within 5 min. The nitrocefin test was negative for *N. cannea*, *N. testacea* and *N. asiatica*, whereas *N. cyriacigeorgica* isolates showed variability in the results of nitrocefin test, with one of the three strains being negative. Positive beta-lactamase *Nocardia* included *N. otitidiscaviarum* and *N. cassinum*, which also exhibited resistance to cefotaxime and imipenem.

**DISCUSSION**

Identification of *Nocardia* to species level using phenotyping is difficult (1,2). Sequencing of the 16S rRNA gene enables more accurate identification, and the application of this technique has resulted in the identification of new and clinically important species of *Nocardia* over the past 10 years (28-30). The number of reported clinical cases caused by opportunistic nocardiosis infections is constantly rising. Seven species of *Nocardia* from 13 cases were reported in a relatively short period during the present study (Table 1). *N. cyriacigeorgica* and *N. farcinica* are the most common species associated with clinical specimens in Qatar, each represented by three cases, collectively constituting 46% of the cases. *N. cyriacigeorgica* is frequently isolated from clinical specimens (31,32). The most frequently reported cases of *N. cyriacigeorgica* constitute disseminated infections with various risk factors including bacteremia in a renal transplant (33), brain abscess in HIV (34), endocarditis (35) and pulmonary infections (36). In the present study, *N. cyriacigeorgica* was isolated from pulmonary infections in two cases and one disseminated infection in a patient with non-Hodgkin’s lymphoma. *N. farcinica* has been reported to be an increasing cause of localized and disseminated infections in immunocompromised patients in recent years (37-39), but bacteremia remains a rare finding (40,41). In the present study, we reported a case of bacteremia in a 50-year-old woman with breast cancer undergoing chemotherapy. The second case was a pulmonary infection in a renal transplant patient, treated successfully with meropenem/trimethoprim-sulfamethoxazole, whereas the third case was conjunctivitis in a patient with no apparent immune dysfunction. Although *Nocardia* infection of any type involving the eye is rare, several species have been diagnosed as a cause of keratitis (42); the isolation of *N. farcinica* in the present study will be added as a possible etiological agent of eye infection. *N. otitidiscaviarum* has been isolated from a fatal brain abscess in a patient with chronic obstructive pulmonary disease (22), in a case of bacteremia (43) and also from pulmonary infection (44); in the present study, this species was found in two cases: corneal abscess in an immunocompetent patient and severe pneumonia in a patient with cystic fibrosis. In a retrospective analysis that included 17 cystic fibrosis patients (18), five *Nocardia* species (including *N. otitidiscaviarum*) were considered as colonizers and oral antibiotic therapy did not appear to affect the clinical outcome. A patient with cystic fibrosis in the present study (case 5) was hospitalized for severe pneumonia that was treated successfully with clarithromycin, trimethoprim-sulfamethoxazole and moxifloxacin with clinical improvement and negative post-treatment sputum culture.

Several rare pathogens were identified in the present study. *N. cannea*, less frequently encountered as a human pathogen (32), was isolated from two cases of pulmonary infections. In one report, the species was isolated from a pulmonary infection in a patient with tuberculosis (45). To our knowledge, *N. cassinum* has not been reported as a human pathogen since its isolation in 1998 from Pacific oysters (46). This species was isolated from a paravertebral abscess at L3-L5 from a patient with a non-Hodgkin’s lymphoma undergoing chemotherapy. *N. testacea*, isolated from sputum of a patient with previous pulmonary tuberculosis in the present study, has been rarely isolated from clinical specimens (47). *N. asiatica*, a rare agent of

**TABLE 2**

Susceptibility pattern of *Nocardia* species to most common antibiotics (μg/mL)

<table>
<thead>
<tr>
<th>Organism (genus)</th>
<th>AK</th>
<th>MOX</th>
<th>CTX</th>
<th>SXT</th>
<th>LIN</th>
<th>IMI</th>
<th>BL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1 <em>N. cannea</em></td>
<td>0.19</td>
<td>0.38</td>
<td>0.016</td>
<td>0.25</td>
<td>0.19</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Case 2 <em>N. cannea</em></td>
<td>4</td>
<td>0.75</td>
<td>0.64</td>
<td>0.38</td>
<td>0.19</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Case 3 <em>N. cannea</em></td>
<td>6</td>
<td>ND</td>
<td>0.19</td>
<td>0.75</td>
<td>0.75</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Case 4 <em>N. otitidiscaviarum</em></td>
<td>3</td>
<td>&gt;256</td>
<td>12</td>
<td>0.75</td>
<td>&gt;32</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Case 5 <em>N. otitidiscaviarum</em></td>
<td>1</td>
<td>&gt;256</td>
<td>12</td>
<td>0.75</td>
<td>&gt;32</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Case 6 <em>N. farcinica</em></td>
<td>2</td>
<td>0.023</td>
<td>1.5</td>
<td>0.064</td>
<td>0.38</td>
<td>0.094</td>
<td>+</td>
</tr>
<tr>
<td>Case 7 <em>N. farcinica</em></td>
<td>2</td>
<td>0.047</td>
<td>6</td>
<td>0.5</td>
<td>0.75</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>Case 8 <em>N. farcinica</em></td>
<td>0.75</td>
<td>0.064</td>
<td>16</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>+</td>
</tr>
<tr>
<td>Case 9 <em>N. cannea</em></td>
<td>0.19</td>
<td>0.38</td>
<td>2</td>
<td>0.5</td>
<td>0.25</td>
<td>0.38</td>
<td>-</td>
</tr>
<tr>
<td>Case 10 <em>N. cannea</em></td>
<td>0.25</td>
<td>0.19</td>
<td>2</td>
<td>0.25</td>
<td>0.094</td>
<td>0.75</td>
<td>-</td>
</tr>
<tr>
<td>Case 11 <em>N. cassina</em></td>
<td>&gt;256</td>
<td>0.38</td>
<td>&gt;256</td>
<td>1.5</td>
<td>0.25</td>
<td>&gt;32</td>
<td>+</td>
</tr>
<tr>
<td>Case 12 <em>N. testacea</em></td>
<td>0.38</td>
<td>0.094</td>
<td>1.5</td>
<td>0.38</td>
<td>0.19</td>
<td>0.25</td>
<td>-</td>
</tr>
<tr>
<td>Case 13 <em>N. asiatica</em></td>
<td>0.094</td>
<td>&gt;32</td>
<td>0.094</td>
<td>0.08</td>
<td>0.032</td>
<td>0.19</td>
<td>-</td>
</tr>
</tbody>
</table>

AK, Amikacin; BL, Beta-lactamase; CTX, Cefotaxime; IMI, Imipenem; LIN, Linezolid; MOX, Moxifloxacin; SXT, Trimethoprim-sulfamethoxazole; – Negative; + Positive

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Figure 3) Chest computed tomography scan of an immunocompetent patient (case 3). A small area of consolidation in the inferior segment of lingual (left lung) caused by *Nocardia cyriacigeorgica* is apparent.
Nocardiosis, was first described in 2004, including five strains isolated from Asia (48), and six documented clinical isolates causing pneumonia or cutaneous infections in patients with HIV and a bone marrow recipient (32), and a disseminated infection in HIV patient (31). It was isolated in the present study from a breast cancer patient with pulmonary infection (case 13). This suggests that, although rarely seen in clinical specimens, N asiatica is associated with immune dysfunction. All patients were treated with appropriate antibiotics and the infection resolved without further sequelae.

The organisms are readily aerosolized with dust and the respiratory tract remains the main portal of entry, with the majority of patients presenting with pulmonary involvement (4,49). Due to its nontypical manifestations, nocardiosis is frequently misdiagnosed; the initial diagnosis is often pneumonia, tuberculosis or lung abscesses. Radiographic presentation may reveal bronchiectasis with pneumonia (Figure 3).

Five immunocompromised patients (Table 1; cases 1, 7, 8, 11 and 13) were successfully treated with the appropriate antibiotics, but case 11 died from a progressive hematological disease. Disseminated nocardiosis, particularly in those with central nervous system involvement or bacteremia, has a poor prognosis with a high mortality rate in immunocompromised hosts (6,17,22,50).

Accurate identification of Nocardia species is important because different species may have different antimicrobial susceptibilities. Linezolid had a distinctive activity pattern against all Nocardia species (Table 2); these results are in accordance with previously document the species prevalent in Qatar.

In the present study, various Nocardia species exhibited different drug susceptibility patterns to cefotaxime and imipenem. The two isolates of N otitidiscaviarum showed a typical multidrug resistance pattern, characterized by resistance to cefotaxime and imipenem and positive for beta-lactamase activity by nitrocefin disk test. Similar resistance patterns were documented in other reports (51-53). N crassostreae was resistant to cefotaxime, imipenem and amikacin; however, amikacin was uniformly active against all other Nocardia isolates (Table 2). Amikacin was demonstrated to be highly active against all tested Nocardia species (51,52). Resistance to amikacin is rare and has been reported mainly for N transvalensi (53,54); because this is the first report for N crassostreae as a human pathogen, susceptibility data were not available in the literature for clinically isolated strains. N farcinica, one of the predominant species in the present study, was susceptible to all agents. Despite exhibiting beta-lactamase activity by nitrocefin disk test, all isolates were susceptible to cefotaxime and imipenem. One strain was intermediately susceptible to cefotaxime. Resistance to cefotaxime and imipenem does not appear to be mediated by beta-lactamase, but rather by decreased affinities of penicillin binding-proteins for these molecules (55).

Among other species, isolates of the N asiatica, N carneae and N tatea have been reported only rarely as human pathogens. N asiatica was resistant to moxifloxacin but susceptible to all other five tested antimicrobial agents. However, few isolates were tested for susceptibility, which showed a resistance pattern for ciprofloxacin (51,53), suggestive of a quinolone resistance profile. This class of antibiotics may not be considered for the treatment of infections caused by this species.

The present study is the first report of series of Nocardia infection, documenting the species prevalent in Qatar. N crassostreae was reported for the first time as a human pathogen. The detection of multidrug resistance species necessitate molecular identification and susceptibility testing, and should be performed for all Nocardia infections. The disease manifests with different clinical features depending on the Nocardia species and underlying conditions. Most patients recovered with combined antimicrobial agents. Trimethoprim-sulfamethoxazole alone or in combination, or sequential with other agents, was effective in treating the majority of patients.

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