CASE REPORT

A case of cutaneous nocardiosis caused by *Nocardia cyriacigeorgica* in a diabetic patient

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Abstract

We present a case of cutaneous abscess with *Nocardia cyriacigeorgica* in a diabetic patient, and review the published work. This patient had infectious abscess on his left lower thigh and the right lower abdomen. The isolated organism was identified by DNA amplification and sequence analyses of the 16S rRNA. After incision and drainage, the clinical application empirically used linezolid and SMZCo took effect.

Introduction

Nocardia species are a group of aerobic actinomycetes, which are filamentous, branching, gram-positive, and weakly acid-fast bacilli¹, higher bacteria widely exist in nature.

Nocardiosis is a not common infection with a variety of clinical manifestations. It mostly affects the lung, brain or skin² in not only immunocompromised but also immunocompetent patients. Herein, we present a diabetic patient probably with low immunity, had cutaneous abscess caused by *Nocardia cyriacigeorgica*. This case was diagnosed by DNA amplification and sequence analyses of the 16S rRNA. We also review cases of nocardiosis in China since the past sixteen years. Then in all the cases of cutaneous nocardiosis in China mainland, our document is the first report of the *N. Cyriacigeorgica* infection.

Case report

A 58-year-old male patient was admitted to our hospital on November 2015, presented with a 9-day history of the left lower extremity swelling. One day before admission, the patient got high fever with the temperature up to 38.4 °C, and left lower extremity reddish swelling with the pain getting worse. Left calf circumference reached 38 cm, which was surrounded relatively thicker than right calf circumference 5 cm. The patient had diabetes, hypertension, hyperlipidemia, hepatitis B, hypoproteinemia, and 3-month history of nephrotic syndrome, but no family heredity case history. He was taking 28 mg qd methylprednisolone and 20 mg qd tripterygium glycosides.

After admission, patient's blood pressure was 97/68 mmHg, pulse rate was 124/min. Laboratory investigations revealed hemoglobin: 119 g/L, total white blood cell count: $19.6 \times 10^9/L$, and differential count: P-88.3%, L-8.7%, M-3%, E-0%, B-0%, platelets: $320 \times 10^9/L$, and with erythrocyte sedimentation rate (ESR) of 90 mm/h. Random blood sugar (RBS) was 11.4 mmol/L, and glycosylated hemoglobin was 8.1%. C-reactive protein (CRP) was 36.3 mg/dL and procalcitonin (PCT) was 14.48ng/ml. Serum total protein was 46.1g/L, albumin was 24 g/L, and globulin was 22.1 g/L. Serology for human immunodeficiency virus (HIV) was negative and for HBV antigens was positive. T-SPOT. TB testing and assay of Plasma (1-3)- β -D-glucan both are negative. The computed tomography (CT) scan of the chest revealed small numbers of nodules distributed on two lungs, and some moist rales could be heard in the lower right lung. Culture of the sputum was *Bauman Acinetobacter*. Blood culture was negative.

Considering the soft tissue infection of the left lower extremity, an topical incision and drainage was the first choice, then drained out dark red paste liquid 70 mL during the process. We had taken the biopsy from ulcer of left lower leg, histopathological examination revealed inflammatory granulation tissue and necrotic tissue.

Suspecting infection, culture tests were conducted by collecting pus and tissue samples from the operative incision of left lower thigh. Microscopic examination revealed a few aerial hyphae in cells (Fig. 1A). Collected pus streaking inoculated on blood agar medium, incubated at 37 °C in 5% CO₂, after 24 h observed results. Tip size, very easily neglected colonies appeared on the plate on day 2. Then,

Fig 1 Pictures. A: Microscopic examination revealed a few aerial hyphae in cells (original magnification $\times 400$); B: White colored colonies in 1 millimeter diameter that grow on the blood agar medium; C: Microscopic examination showed the gram stained positive branched bacilli (original magnification $\times 400$); D: Modified acid-fast staining showed the partial acid-fast branched bacilli (original magnification $\times 400$).

white gypsum-colored colonies in 1 millimeter diameter that emitted an earthy odor began to grow on the plate on day 3 (Fig. 1B). The colony was dry, rough, edged into the surface of the medium, and was difficult to be provoked or push. Gram staining identified Gram-positive branched mycelia bacilli (Fig. 1C). Modified acid-fast staining showed partial acid-fast branched bacilli (Fig. 1D).

Biochemical identification tests revealed the organism to be catalase positive, but indole, lysine and ornithine decarboxylase tests were negative, could broke down esculin but not urea, could not decompositive casein, hypoxanthine, xanthine or tyrosine. Acid formation was confirmed from fructose, but not from glucose, lactose, maltose, sucrose, inositol, rhamnose, sorbitol or mannitol.

Identification using the VITEK 2 fluorescent system identified uncultured bacterium. The organism were confirmed by DNA amplification and sequence analyses of the 16S rRNA, a GenBank Basic Local Alignment Search Tool (BLAST) search revealed that the 16S rRNA gene sequence of this isolate showed 100% homology corresponding sequences of the previously sequenced of *N. cyriacigeorgica* ATCC14759. Based on the results of various tests, the patient was diagnosed with cutaneous abscess nocardiosis caused by *N. cyriacigeorgica*.

In terms of drug susceptibility, this isolate was sensitive to the trimethoprim-sulfamethoxazole (TMP-SMZ), linezolid, minocycline, amikacin, imipenem, amoxycillin/clavulanate, cefuroxime, cefodizime, cefotaxime, gentamycin, levofloxacin and etimicin. Meanwhile, the isolate was resistant to vancomycin, teicoplanin, doripenem, meropenem, penicillin, oxacillin, clindamycin, ceftriaxone, ceftazidime and erythromycin. (Susceptibility testing was performed according to CLSI document M24-A. If there is no antibiotic sensitive break point, we judge according to the corresponding break point of *Staphylococcus*.)

Prior to the isolation of *Nocardia*, the patient was empirically treated by cefodizime and metronidazole, high fever turned to intermittent fever, and a 2 cm, hard subcutaneous mass was discovered on the patient's right lower abdomen. Once the diagnosis of nocardiosis was made, according to the availability of drug susceptibility results, the treatment was switched to intravenous injection of 0.6 g linezolid every 12 h, and oral administration of 0.1 g minocycline together with 2 g SMZCo every 12 h, the wound treated with silver sulfadiazine cream. The patient's body temperature and white blood cell drop to normal level, monitoring renal function was also normal. The incision of left lower thigh healed well, and the subcutaneous mass

on the right lower abdomen was gradually absorbed. The patient felt in good condition, then applied for discharge recuperation from hospital.

Discussion

Nocardiosis is an opportunistic infection that mainly affects immunocompromised hosts, especially individuals with impaired cell-mediated immunity associated with malignant tumour, transplantation, glucocorticoid therapy, leukemia, systemic lupus erythematosus (SLE) and acquired immune deficiency syndrome (AIDS). However, this infection also can occur in immunocompetent patients as previously reported.³ The clinical manifestations of nocardiosis merely involve the lungs in many cases; however, *Nocardia* bacteria can also disseminate from the lungs to any other organ as a consequence of hematogenous spread, commonly involves the central nervous system, skin soft tissue, kidney, pleura, pericardium, bone and joint. *Nocardia* is an opportunistic pathogen, not human body normal flora, so there will not be an endogenous infection.

Eppinger reported the first human *Nocardia* sp. infection in a patient with pneumonia and brain abscess in 1890⁴, and so far hundreds of species of *Nocardia* sp. have been found. According to the medical literatures in China that still can be completely consulted, Shouzheng H and Zhenying Z reported the first *Nocardia* sp. infection in a patient with brain abscess in 1979.⁵ That report is the first one to detect *N. asteroides* from a brain abscess sample secondary to finger trauma in our country. Retrospective analysis of *Nocardia* infection cases in China from 2000 to 2016, approximately 272 cases have been completely reported, and their ages range 4 days old to 85 years old. Percentage by sex: male accounting for 58.0%, female accounting for 41.4%. With regard to location, 154 patients (56.6%) had pulmonary nocardiosis (including patients with other lesions), 76 of the 154 patients had only pulmonary lesions. 115 patients (42.3%) had skin and soft tissue infection symptoms, while infection of joint (6 patients) and ocular (10 patients), basically due to trauma or surgical infection, have also been described. What's more, there have been group occurrence of cutaneous nocardiosis reported in China and abroad have not seen such reports.

With regard to identification approaches, just 16 patients (5.9%) with positive blood culture, 1 patient with positive urine culture and 2 patients with positive vaginal secretion culture which are very rare. The remaining cases were diagnosed by sputum culture or abscess secretion culture. With regard to strains, *Nocardia asteroides* was most common

(110 cases, accounting for 40.4%), followed by *Nocardia brasiliensis* (31 cases, accounting for 11.4%), then 103 isolates (37.9%) were not identified to species. With regard to drug tolerance, 199 isolates (73.2%) were sensitive to TMP-SMX and 8 isolates with drug resistance. After treatment, 211 cases (77.6%) improved and 25 cases (9.2%) died.

In the course of our literature review, it shows that the immunocompetent patient infection is usually caused by a wound infection or breathing in dust containing bacterium under the special environment, however, the Nocardiosis is still a kind of infectious disease always occur in immune deficiency patients. There are 154 patients (56.6%) with underlying disease, 108 cases of them used of glucocorticoids or immunosuppressive agents. The most common diseases included systemic lupus erythematosus, type II diabetes mellitus, chronic obstructive pulmonary disease, nephrotic syndrome and AIDS. Of particular note is, preexisting pulmonary diseases such as chronic obstructive pulmonary disease (COPD) and bronchiectasis also are additional risk factors for nocardiosis, which occur when bacterial colonization in the lower respiratory tract alters ciliary motility and causes epithelial damage⁶, but the mechanism of this risk factor has not been completely clear yet. Therefore, the patients with systemic lupus erythematosus disease, chronic pulmonary disease, COPD, renal disease, and using corticosteroids or immunosuppressant, once get fever, cough, shadows in the lung, pleural effusion or pulmonary cavity, especially the emergence of skin and intracranial disseminated lesions, are required to alert *Nocardia* infection. Most of the time, pulmonary nocardiosis and common lung infection is difficult to distinguish. Chest computed tomography (CT) findings in *Nocardia* species infection often resemble those in common lung diseases, thus, nocardiosis is often misdiagnosed as pneumonia, tuberculosis, histoplasmosis, actinomycosis, fungal infections, bacterial abscess etc. Further more, it has been reported that though the clinical features of the patient were mainly related to the respiratory system without neurological symptom and sign, radiological imaging still showed cerebral abscesses, suggesting that the neurological and other systems should be routinely examined on the pulmonary nocardiosis patients to rule out the possible infections and to provide guidance for therapeutic plans.

During the treatment process, trimethoprim-sulfamethoxazole has always been the first choice for the use of antibiotics, usually in combination with one or two other sensitive antibiotics to achieve satisfactory therapeutic effect. Some patients with renal dysfunction, or in the course of the

using SMZCo have digestive tract symptoms and can not be tolerated, may replace with Amikacin, imipenem, third-generation cephalosporins, minocycline, netilmicin, and amoxicillin/clavulanic acid which are also effective against *Nocardia* isolates *in vitro*.⁶ Skin and soft tissue infections in addition to medication, should combine with drainage and debridement.

N. cyriacigeorgica was first characterized in 2001 by Yassin et al. as a novel species, the isolate with a drug pattern type VI was determined by 16S rRNA and 65-kDa heat shock protein polymerase chain reaction and sequence analysis to be a new species named *N. cyriacigeorgica*. In China, Yuanyong TAO reported the first *N. cyriacigeorgica* disease in a patient with pulmonary infection in 2007. To the best of our knowledge, in all the cases of cutaneous nocardiosis in China mainland, our document is the first report of the *N. Cyriacigeorgica* infection. Afterwards, a recent study showed that *N. cyriacigeorgica* is the most frequently isolated strain in Taiwanese patients with pulmonary nocardiosis (10 of 20 cases).⁷ Because it is not easy to identify the bacteria just depending on the phenotypic characters, while many clinical laboratories do not have the conditions for molecular biology experiments, part of the *N. cyriacigeorgica* may be undetected or misjudged into other bacteria.

Due to *Nocardia* strains is difficult to culture, and the lack of specific clinical manifestations of infection, diagnosis of nocardiosis is very inconvenient. Common identification methods include: staining, biochemical characterization, and molecular biological methods. The Gram stain positive characteristic branching bacilli, and acid-fast stain negative, weak acid-fast stain positive for bacilli, can be used as the basis for the identification of *Nocardia*. But the results can only be used as an auxiliary diagnosis, and can not be considered as the final diagnosis. Biochemical identification method can not get the desired results every time to quickly identify *Nocardia*, not to mention bacterial typing.

However, the advent of molecular methods has led to a better evaluation of patients with uncommon, difficult-to-diagnose infections such as nocardiosis. Utilizing DNA sequences and in particular construction of 16s rRNA libraries have had great advantage on the enhancement of species identification within the genus *Nocardia*. Our laboratory amplified the 16S rRNA gene with primers BAK11w[5-AGTTTGATC(A/C)TGGCTCAG] and BAK2[5-GGACTAC(C/T/A)AGGGTATCTAAT]. Cycling parameters included an initial denaturation for 5 min at 95 °C; 40 cycles of 1 min at 94 °C, 1 min at 48 °C, and

1 min at 72 °C; and a final extension for 10 min at 72 °C.⁸ These primers and reaction conditions are also successfully used to identify other rare gram positive coccus and bacillus in our laboratory, such as *Rhodococcus* and *Bifidobacterium*, provides high accuracy, convenience and high measurement speed.

Nocardia infection localized disease general prognosis is good. The patients with complicated infection, spread of bacteria and poor condition of foundation usually get the poor prognosis and higher mortality rate. Early diagnosis and treatment, the application of sensitive drugs are critically important for improving the prognosis and reducing the mortality rate of patients. We suggest that the laboratory personnel and clinical doctors should strengthen communication in routine work, to prompt the microbial inspection personnel adjust the culture conditions and extend the time of incubation, for the sake of improving the detection rate of rare bacteria.

Declarations

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No.

Competing interests

The authors declare that they have no competing interest.

Authors' contributions

YC Jia and SD Song made the literature analysis and wrote, discussed and revised the manuscript of this review. XM Wu and W Qi critically analyzed and corrected the manuscript. All authors read and approved the final manuscript.

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