

A post-transplant infection by *Nocardia cyriacigeorgica*

Nageswari Gandham, Sriram Kannuri, Aryan Gupta, Sahjid Mukhida, Nikunja Das and Shahzad Mirza*

Abstract

Nocardia are Gram-positive, acid-fast, filamentous bacteria that cause opportunistic infections in susceptible populations. We describe a case of post-transplant infection of pulmonary nocardiosis caused by the rare strain *Nocardia cyriacigeorgica* and the challenges faced in reaching a definitive diagnosis. This case report emphasizes on keeping nocardiosis as a differential diagnosis in transplant recipients, as this disease is largely underdiagnosed and underreported.

DATA SUMMARY

No data was generated or reused.

INTRODUCTION

In patients receiving immunosuppressive therapy after renal transplantation, the possibility of opportunistic infections increases several fold. One such life-threatening opportunistic infection is nocardiosis [1], caused by organisms that are mainly inhabitants of water and soil [2]. Among the various species causing the said disease, *Nocardia asteroides* complex is most common, followed by *Nocardia brasiliensis* and *Nocardia otitidiscaviarum* [3, 4].

Looking at the spectrum of diseases caused by *Nocardia*, it most often involves the respiratory system, but it also shows affinity towards the brain, skin and soft tissues [5]. Although it rarely affects immunocompetent patients, a positive case is most likely a result of inoculation of the organism directly through foreign object penetration into the body [4]. Incidence of nocardiosis after solid organ transplant is rare in comparison to other opportunistic infections, amounting to only ~0.6% [6]. T-cell depleting therapies, including antithymocyte globulin, increase the risk for opportunistic infection. Infected patients mostly present with signs and symptoms of lower respiratory tract infections that do not respond to routine empirical antibiotics, along with progressive deterioration in the general condition of the patient [2]. Microbiological diagnosis of such cases can be tricky given the paucity of organisms in respiratory samples after ruling out other common causes of the infections such as tuberculosis, bacterial pneumonia and invasive fungal disease. Traditional techniques along with recent advancements in microbiology can be combined to reach an accurate diagnosis in such cases [7]. Here, we highlight a case of pulmonary nocardiosis in an immunocompromised patient post-renal transplantation caused by a rare species, *Nocardia cyriacigeorgica*.

CASE PRESENTATION

A 41-year-old male with a history of grade 5 chronic kidney disease, diagnosed 2 years ago, had an HLA 6/6 mismatch living-related renal transplant: wife to husband in the month of April 2022. Post-transplant, the patient was put on immunosuppressive medications. Approximately 2 months after the transplant, the patient gradually developed fever, which was intermittent in nature, and a cough with expectoration, which exacerbated on supine position. To evaluate the aetiology, sputum and broncho-alveolar lavage (BAL) sample collection was done, and the specimens were sent for microbiological investigation. Chest X-ray, ultrasonography of the thorax and high-resolution computed tomography (HRCT) revealed bilateral nodular consolidations that were progressive in nature (Figs 1 and 2). The patient's sputum and BAL culture was negative for bacteria, fungi and *Mycobacterium tuberculosis*. As a last resort, a closed transbronchial lung biopsy was done given the insignificant sputum and

Received 23 January 2023; Accepted 24 October 2023; Published 08 November 2023

Author affiliations: ¹Department of Microbiology, Dr. D. Y. Patil Medical College, Hospital and Research Centre, Dr. D. Y. Patil Vidyapeeth University, Pune, Maharashtra, India.

***Correspondence:** Shahzad Mirza, drshahzadmira83@gmail.com

Keywords: *Nocardia cyriacigeorgica*; nocardiosis; opportunistic infection; renal transplantation.

Abbreviations: BAL, broncho-alveolar lavage; MALDI-TOF, matrix-assisted laser desorption/ionization-time of flight; ZN, Ziehl-Neelsen.

000569.v3 © 2023 The Authors



This is an open-access article distributed under the terms of the Creative Commons Attribution License.

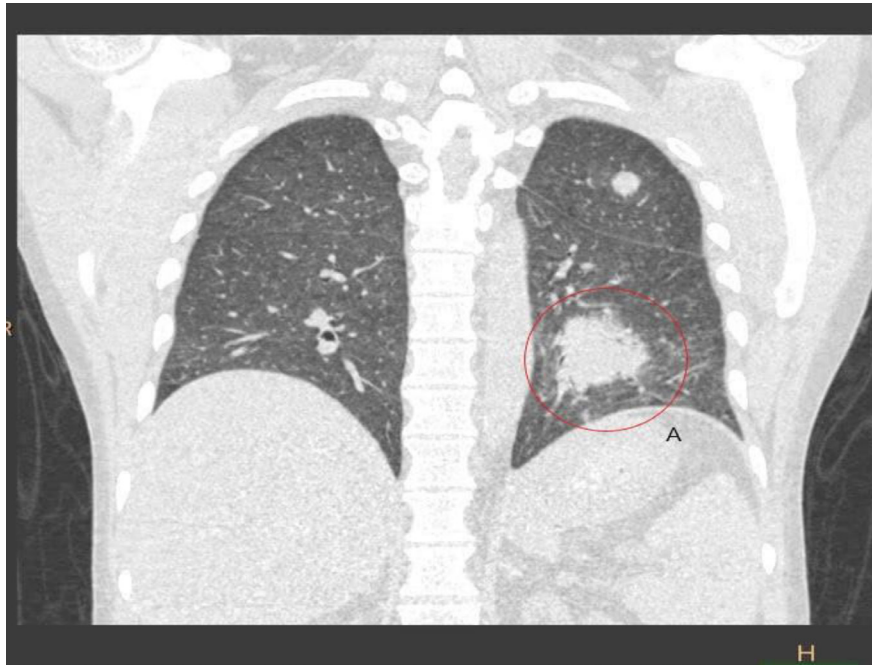


Fig. 1. Coronal section of computed tomography scan showing multiple nodules and mass-like consolidations in the left lung. A encircles the largest consolidation.



Fig. 2. Axial section of computed tomography scan. B encircles the largest consolidation in the left lung.

BAL studies, and progressive deterioration in the condition of the patient. A Gram-stain smear of excised tissue exhibited a Gram-positive branching filamentous structure, and Ziehl–Neelsen (ZN) stain of the same revealed a weak acid-fast branching filamentous structure. A modified acid-fast staining using 0.5–1% sulphuric acid as a decolourizer (rather than the conventional 20% sulphuric acid) serves as an extremely useful guide for the diagnosis of *Nocardia*, aligning with the observations of Gram-stain and ZN stain. The modified ZN revealed acid-fast branching filamentous bacilli. The inoculation of the tissue sample was

done on blood, chocolate and MacConkey's agar. On blood and chocolate agar, dry, chalky white, medium-sized colonies were observed, whereas no colonies were observed on MacConkey's agar. To observe the gross appearance in liquid media, tissue was also inoculated into Robertson's cooked meat (RCM) broth, in which large, chalky white pellicles were found. Smears from the colonies of both blood and chocolate agar were subjected to Gram, ZN and 1% modified ZN staining, and subsequent microscopy revealed a Gram-positive, acid-fast, branching, filamentous bacilli. The colonies were also inoculated onto Mueller-Hinton agar by lawn technique to check for drug susceptibility, as a method to determine the possible group of *Nocardia* causing the condition (Fig. 3). Drugs used for differentiation by susceptibility were tobramycin, amikacin, gentamicin and erythromycin [7]. The cultured isolate was found to be susceptible to tobramycin, amikacin and gentamicin, but was resistant to erythromycin

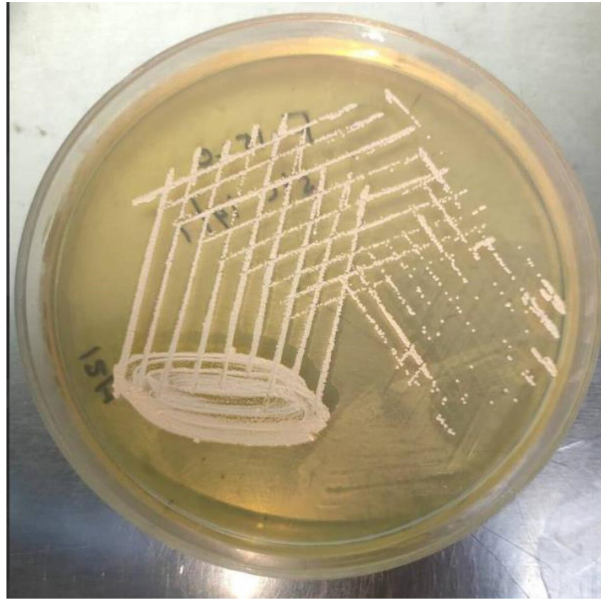


Fig. 3. Growth of colonies of the isolate on Mueller-Hinton agar.

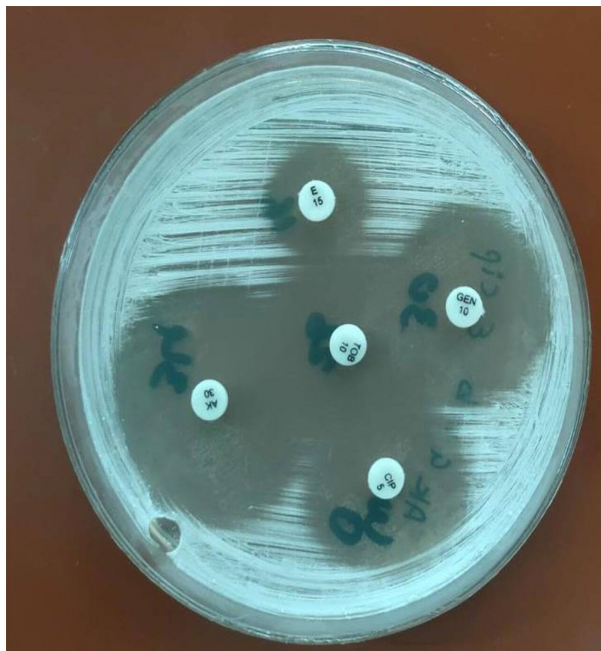


Fig. 4. *Nocardia* spp. susceptibility to amikacin (Ak), gentamicin (Gen), tobramycin (Tob) and ciprofloxacin (Cip).

(Fig. 4). Based on this pattern of susceptibility, it was suspected that the organism was among the following, i.e. *N. asteroides* I/VI, *N. brasiliensis*, *Nocardia pseudobrasiliensis* and *N. otitidiscaviarum*. For complete identification of the species, a MALDI-TOF (matrix-assisted laser desorption/ionization-time of flight) MS ID test (MALDI Biotyper Sirius; Bruker) was performed on the sample. The isolate was identified as *N. cyriacigeorgica*. The patient was started on injection meropenem 1 g, twice daily for 10 days, and oral co-trimoxazole once daily (sulfamethoxazole 800 mg/trimethoprim 160 mg) for 25 days, and further was put on prophylaxis with oral co-trimoxazole – 200 µg for 3 weeks. The patient's response to treatment was favourable. Follow-up for the patient was uneventful and subsequently a lung biopsy was done for confirmation (as the patient was immunocompromised and to avoid further complications), which revealed no similar bacterial culture.

DISCUSSION

Nocardia species are known to be Gram-positive, filamentous, branching bacilli that are also partially acid-fast. The prevalence of nocardiosis is rare in comparison to other bacterial infections; however, under certain conditions, it should be kept as a differential diagnosis that should not be ignored [4]. The incidence of *Nocardia* infections after a solid organ transplant can be between 0.04 and 3.5% [8], and the incidence in renal transplant patients can be between 0.04 and 1.2% [9]. According to a case-control study conducted by Peleg *et al.*, the risk factors for nocardiosis in solid organ transplant recipients can be attributed to high-dose steroid therapy, infection with cytomegalovirus (CMV) and high median calcineurin inhibitor levels in the preceding 30 days (>300 ng ml⁻¹ for cyclosporine and >15 µg ml⁻¹ for tacrolimus) [6]. It was also noted that patients receiving tacrolimus were at a higher risk of contracting nocardiosis than those receiving cyclosporine [10]. Our patient also had tacrolimus trough levels of 6 µg ml⁻¹ and high-dose corticosteroids to avoid organ rejection due to HLA mismatch. It is reported that *Nocardia* infections usually present within a period of 6 months and rarely 1 year post-transplant, similarly our patient presented with the typical symptoms of cough, fever and breathlessness [3]. Due to the lack of distinctive clinical findings or radiological evidence indicative of a *Nocardia* infection, Gram-staining of sputum samples remains a vital tool for diagnosis [3, 5, 7]. Bilgarnia *et al.* and Flohr *et al.* reported two other cases of pulmonary nocardiosis. Both cases underwent re-transplantation, and preconditioning was done using plasmapheresis and rituximab. Post-transplant, these patients were maintained on mycophenolate mofetil, tacrolimus and steroids [11, 12]. We received multiple sputum specimens, but all their Gram-stain reports were insignificant and ZN staining was negative for the presence of acid-fast bacilli. For better diagnostic sensitivity, BAL samples were then taken, which proved to be a failure. As prior investigations proved to be inconclusive, more invasive techniques were indicated [13]. Invasive samples like BAL were sent, which were negative for infections like mycobacterium, but because of the persistent radiological evidence, a lung biopsy was done that proved *Nocardia* species, an opportunistic infection. The lung biopsy samples assisted in isolating the causative organism by providing the above-mentioned picture in staining and culture. Antibiotic susceptibility for *Nocardia* can be identified by broth microdilution [14], or by the Kirby-Bauer disc diffusion method [15] (Table 1). In our study, broth microdilution was not done due to technical reasons. An accurate diagnosis was made because of the cultures; after which, treatment was initiated promptly to deal with infection. MALDI-TOF MS test, wherever applicable, can be used to diagnose such cases, as it gives fast and reliable results for such difficult-to-diagnose pathogens [16]. Prophylactic treatment for *Nocardia* usually involves long-term co-trimoxazole. Alternatively, drugs such as amikacin, imipenem, meropenem and third-generation cephalosporins have been shown to have efficacy against *Nocardia in vitro* [17].

Table 1. Various case reports dealing with *Nocardia* species isolation and their findings

No.	Authors	Brief description of patient/illness	Species isolated	Treatment	Antibiotic-susceptibility pattern by Kirby-Bauer disc diffusion method	Antibiotic-susceptibility pattern by broth microdilution method
1	Khadka & Shah [15]	Cutaneous nocardiosis post-renal transplant	<i>Nocardia asteroides</i>	Trimethoprim/sulfamethoxazole	Yes.	No
2	Zintgraff <i>et al.</i> [14]	Brain abscess in an immunocompromised patient	<i>Nocardia farcinica</i>	Trimethoprim/sulfamethoxazole and linezolid	No	Yes
3	Rodríguez-Lozano <i>et al.</i> [18]	Post-traumatic endophthalmitis	<i>Nocardia nova</i>	Amikacin, imipenem	Yes (by Etest strips; bioMérieux)	No
4	Kuchibiro <i>et al.</i> [19]	Pulmonary nocardiosis	<i>Nocardia mexicana</i>	Trimethoprim/sulfamethoxazole	No	Yes
5	Shokri <i>et al.</i> [20]	Pulmonary and cutaneous nocardiosis	<i>Nocardia mexicana</i>	Linezolid/amikacin	No	Yes

Conclusion

Amongst a plethora of opportunistic infections affecting immunocompromised patients, *Nocardia* should be kept as a differential diagnosis in renal transplant recipients presenting with a pulmonary infection. Invasive diagnostics can help the patient out with an early diagnosis of nocardiosis and prevent disease dissemination. If needed, the use of advanced techniques such as MALDI-TOF MS should be sought for an early and reliable diagnosis.

Funding Information

The authors received no specific grant from any funding agency.

Author contributions

N.G.: conceptualization, validation, supervision, project administration. S.K.: investigation, writing – review and editing. A.G.: investigation, writing – review and editing. S.Muk.: investigation, writing – review and editing. N.D.: conceptualization, methodology, validation, supervision, project administration. S.Mir.: conceptualization, methodology, validation, investigation, writing – original draft (lead), software (search strategy), supervision, project administration.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical statement

Ethical approval was obtained from the Institutional Ethics Committee (IEC) of the university.

Consent to publish

The authors declare that consent for publication has been obtained.

References

- Gibson M, Yang N, Waller JL, Young L, Bollag WB, et al. Nocardiosis in renal transplant patients. *J Investig Med* 2022;70:36–45.
- Beaman BL, Beaman L. *Nocardia* species: host-parasite relationships. *Clin Microbiol Rev* 1994;7:213–264.
- Saubolle MA, Sussland D. Nocardiosis: review of clinical and laboratory experience. *J Clin Microbiol* 2003;41:4497–4501.
- Duggal SD, Chugh TD. Nocardiosis: a neglected disease. *Med Princ Pract* 2020;29:514–523.
- Brown-Elliott BA, Brown JM, Conville PS, Wallace RJ Jr. Clinical and laboratory features of the *Nocardia* spp. based on current molecular taxonomy. *Clin Microbiol Rev* 2006;19:259–282.
- Peleg AY, Husain S, Qureshi ZA, Silveira FP, Sarumi M, et al. Risk factors, clinical characteristics, and outcome of *Nocardia* infection in organ transplant recipients: a matched case-control study. *Clin Infect Dis* 2007;44:1307–1314.
- Kiska DL, Hicks K, Pettit DJ. Identification of medically relevant *Nocardia* species with an abbreviated battery of tests. *J Clin Microbiol* 2002;40:1346–1351.
- Lebeaux D, Morelon E, Suarez F, Lanternier F, Scemla A, et al. Nocardiosis in transplant recipients. *Eur J Clin Microbiol Infect Dis* 2014;33:689–702.
- Coussement J, Lebeaux D, van Delden C, Guillot H, Freund R, et al. Nocardia infection in solid organ transplant recipients: a multicenter European case-control study. *Clin Infect Dis* 2016;63:338–345.
- Canet S, Garrigue V, Bismuth J, Chong G, Lesnik A, et al. La nocardiose est-elle plus fréquemment observée depuis l'introduction des nouveaux immunosuppresseurs en transplantation rénale? [Nocardiosis – is it frequently observed after the introduction of new immunosuppressive agents in renal transplantation?]. *Nephrologie* 2004;25:43–48.
- Biglarnia A-R, Wadström J, Tufveson G, Eriksson B-M. Pulmonary nocardiosis with brain abscess in a sensitized kidney transplant recipient with a history of repeated graft loss and HLA-antibody depletion treatment – a case report. *J Med Sci* 2008;113:111–116.
- Flohr TR, Sifri CD, Brayman KL, Hagspiel KD, Sawyer RG, et al. Nocardiosis in a renal transplant recipient following rituximab preconditioning. *Ups J Med Sci* 2009;114:62–64.
- Jorna T, Taylor J. Disseminated *Nocardia* infection in a renal transplant patient: the pitfalls of diagnosis and management. *BMJ Case Rep* 2013;2013:bcr2012007276.
- Zintgraff J, Prieto M, Peña M, Simoiz F, Rosenblit S, et al. When reporting *Nocardia* spp is not enough. Brain abscess caused by *Nocardia farcinica*. *Access Microbiol* 2020;2:acmi000091.
- Khadka P, Shah DS. Primary cutaneous nocardiosis: a diagnosis of consideration in a renal transplant recipient. *BMC Clin Pathol* 2018;18:8.
- AlMogbel M, AlBolbol M, Elkhizzi N, AlAjlan H, Hays JP, et al. Rapid identification of *Nocardia cyriacigeorgica* from a brain abscess patient using MALDI-TOF-MS. *Oxf Med Case Reports* 2020;2020:maa088.
- Martínez Tomás R, Menéndez Villanueva R, Reyes Calzada S, Santos Durantez M, Vallés Tarazona JM, et al. Pulmonary nocardiosis: risk factors and outcomes. *Respirology* 2007;12:394–400.
- Rodríguez-Lozano J, Armiñanzas Castillo C, Ruiz de Alegría Puig C, Ventosa Ayarza JA, Fariñas MC, et al. Post-traumatic endophthalmitis caused by *Nocardia nova* JMM Case Rep 2019;6:e005175.
- Kuchibiro T, Ikeda T, Nakanishi H, Morishita Y, Houdai K, et al. First case report of pulmonary nocardiosis caused by *Nocardia mexicana*. JMM Case Rep 2016;3:e005054.
- Shokri D, Motalebirad T, Jafarinia M, Azadi D, Ghaffari K. First case report of pulmonary and cutaneous nocardiosis caused by *Nocardia mexicana* in Iran. *Access Microbiol* 2019;1:e000016.

Peer review history

VERSION 2

Editor recommendation and comments

<https://doi.org/10.1099/acmi.0.000569.v2.1>

© 2023 Duggan S. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License.

Seána Duggan; University of Exeter, MRC Centre for Medical Mycology, UNITED KINGDOM

Date report received: 24 October 2023

Recommendation: Accept

Comments: The revision of this case report has taken the comments from the peer reviewers into consideration and as a result the manuscript is strengthened. I'm pleased to accept this report for publication.

Author response to reviewers to Version 1

Reviewer 1:

a. Developed fever instead of 'a fever'

Line 89: grammatical error corrected as requested by reviewer 1.

b. Exacerbated on supine position instead of supination

Line 90: sentence rephrased as requested by reviewer 1.

c. Can remove - As a part of evaluation CXR, USG and CT were done. Instead, can write CXR and USG findings

Line 92/93: sentence rephrased as requested by reviewer 1, included chest xray and USG findings

d. Can mention what all sputum and BAL investigations were done and negative (bacterial culture, fungal culture, TB, others)

Line 94: mentioned what all sputum and BAL investigations were done as requested by reviewer

e. How was lung biopsy done? Open lung biopsy or other method?

Line 95: A closed, transbronchial lung biopsy was done. The change has been made, as requested by reviewer 1.

f. Why was a repeat lung biopsy was done when patient was clinically improving ?

Line 121/122: The repeat lung biopsy was done for confirmation, as the patient was immunocompromised, and in order to avoid further complication. The change has been made in the manuscript.

g. Was the patient put on prophylaxis for nocardiosis after clinical recovery/cure?

Line 119: The patient was put on prophylaxis with Co-trimoxazole. This change has been made in the manuscript.

Reviewer 1 comments for the discussion section of the manuscript:

a. Tacrolimus levels - units in ng/mL

Line 134: **NO CHANGE.** We request reviewer 1 to cross check the reference number 6 from our reference section of manuscript, since the unit mentioned in our manuscript has been scientifically published before. You may find more information for the units in the "clinical data" section on this link (<https://academic.oup.com/cid/article/44/10/1307/355675>).

Line 136: After the above comment, it was brought to our notice that the tacrolimus trough levels have been mentioned in ng/mL, which has been corrected in the manuscript to ug/mL.

Reviewer 1 comments for the Figure section of the manuscript:

a. Figure 1 - Legend A encircles the largest nodule - it should be consolidation

Line 234: correction has been made in the manuscript as requested by reviewer 1.

End of changes requested by Reviewer 1

Reviewer 2:

Line 26: The bacteria in the genus Nocardia are filamentous. It recommends using the appropriate word because they are not true bacilli.

Line 26: As suggested by reviewer 2, we have changed the terminology used in Line 26. We have now referred nocardia as gram positive, acid fast filamentous structures.

Line 96: Instead of " A 1% modified ZN... ", show how to do modified acid-fast staining correctly. Take into account that most Nocardia species are partially acid-fast when using modified acid-fast staining like Kinyoun method.

Line 99/100: As requested by reviewer 2, the change has been implemented in Line 99/100..

- The history of medical interventions, including each immunomodulation, medicine, and any other patient's risk factors, should be provided in the case presentation section.

Line 88: As requested by reviewer 2, changes have been made in Line 88.

Patient's immunomodulation has been discussed in the "discussion section" of the manuscript.

- Since the isolate has already been identified using the accurate MALDI-TOF method, it is not required to describe a non-specific identification method like the susceptibility pattern to four antibiotics.

In order to come to a stepwise, systematic diagnosis for Nocardia, we have described our thinking process. As we wanted to narrow down the species, the susceptibility pattern to antibiotics was used.

End of changes requested by Reviewer 2

Reviewer 3:

1. Description of the case(s): Good description of case but can be done better by including some diagnostic parameters that support the evidence of infection by an opportunistic microorganism.

The patient was immunocompromised following a renal transplantation, and presented with symptoms of an infection. Patient was having a persistent cough, and radiological investigation was revealing a consolidation (supporting evidence of this has been attached in FIGURE 1 and 2). After ruling out the endemic causes of such a presentation (TB), a lung biopsy was done, and an opportunistic infection was diagnosed.

2. Presentation of results: The results can be presented in a better fashion by presenting the diagnostic parameters along the course of hospital stay, showing improvement on diagnosis by department of microbiology and how the antibiotic susceptibility helped the treatment of the individual.

The authors feel that the results are being presented in best possible way, as per our resources and expertise. We have made some overall changes in the manuscript to depict the change in patients condition. Invasive samples like BAL were sent which were negative for infections like mycobacterium, but because of the radiological evidence, a lung biopsy was done, which proved Nocardia species, an opportunistic infection. An accurate diagnosis was made because of the cultures, after which, treatment was initiated promptly to deal with nocardia infection. The patient subsequently improved on co-trimoxazole.

3. How the style and organization of the paper communicates and represents key findings. The organization of the paper is good and represents most of the key features.

No changes

4. Literature analysis or discussion: Not up to the mark. Please discuss more cases with their antibiotic susceptibility patterns and please provide a substantial reason as to why broth micro dilution was not performed for the isolate.

Line 153/154: Broth microdilution was not done due to technical reasons.

5. Any other relevant comments: please improve the discussion section as very few studies discussing the antibiotic susceptibility and other cases of infections with the same isolate. Please provide reason for not performing broth micro dilution

Multiple changes to the discussion section has been made. Broth microdilution was not done due to technical reasons. A table mentioning the methods used for antibiotic sensitivity testing for nocardia species by Kirby bauer and broth microdilution has been added.

End of changes requested by Reviewer 3

VERSION 1

Editor recommendation and comments

<https://doi.org/10.1099/acmi.0.000569.v1.6>

© 2023 Duggan S. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License.

Seána Duggan; University of Exeter, MRC Centre for Medical Mycology, UNITED KINGDOM

Date report received: 25 July 2023

Recommendation: Major Revision

Comments: The reviewers indicated further work is necessary to improve the manuscript for publication. Please address all reviewer comments in a point-by-point response, paying specific attention to the following points: clarity and information is required on the diagnostic parameters, patient history including any interventions and medicines, and the antibiotic susceptibility interpretation and comparison to CLSI standards.

Reviewer 3 recommendation and comments

<https://doi.org/10.1099/acmi.0.000569.v1.3>

© 2023 Kar M. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License.

Mitra Kar; SGPGIMS: Sanjay Gandhi Post Graduate Institute of Medical Sciences, Microbiology, A-14, Swapnil Sangam near bijnour road near bijnour primary school behind bijnou, Sarvan nagar, 226002, Lucknow, INDIA

<https://orcid.org/0000-0001-9744-9653>

Date report received: 24 July 2023

Recommendation: Major Revision

Comments: 1. Description of the case(s): Good description of case but can be done better by including some diagnostic parameters that support the evidence of infection by an opportunistic microorganism. 2. Presentation of results: The results can be presented in a better fashion by presenting the diagnostic parameters along the course of hospital stay, showing improvement on diagnosis by department of microbiology and how the antibiotic susceptibility helped the treatment of the individual. 3. How the style and organization of the paper communicates and represents key findings. The organization of the paper is good and represents most of the key features. 4. Literature analysis or discussion: Not up to the mark. Please discuss more cases with their antibiotic susceptibility patterns and please provide a substantial reason as to why broth micro dilution was not performed for the isolate. 5. Any other relevant comments: please improve the discussion section as very few studies discussing the antibiotic susceptibility and other cases of infections with the same isolate. Please provide reason for not performing broth micro dilution

Please rate the quality of the presentation and structure of the manuscript

Satisfactory

To what extent are the conclusions supported by the data?

Partially support

Do you have any concerns of possible image manipulation, plagiarism or any other unethical practices?

No

Is there a potential financial or other conflict of interest between yourself and the author(s)?

No

If this manuscript involves human and/or animal work, have the subjects been treated in an ethical manner and the authors complied with the appropriate guidelines?

Yes

Reviewer 2 recommendation and comments

<https://doi.org/10.1099/acmi.0.000569.v1.5>

© 2023 Anonymous. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License.

Anonymous.

Date report received: 21 July 2023

Recommendation: Minor Amendment

Comments: Dear authors, I read the manuscript in details. Here are some recommendations for improving the manuscript:
 - Line 26: The bacteria in the genus *Nocardia* are filamentous. It recommends using the appropriate word because they are not true bacilli. -Line 96: Instead of " A 1% modified ZN...", show how to do modified acid-fast staining correctly. Take into account that most *Nocardia* species are partially acid-fast when using modified acid-fast staining like Kinyoun method. - The history of medical interventions, including each immunomodulation, medicine, and any other patient's risk factors, should be provided in the case presentation section. - Since the isolate has already been identified using the accurate MALDI-TOF method, it is not required to describe a non-specific identification method like the susceptibility pattern to four antibiotics.

Please rate the quality of the presentation and structure of the manuscript

Satisfactory

To what extent are the conclusions supported by the data?

Strongly support

Do you have any concerns of possible image manipulation, plagiarism or any other unethical practices?

No

Is there a potential financial or other conflict of interest between yourself and the author(s)?

No

If this manuscript involves human and/or animal work, have the subjects been treated in an ethical manner and the authors complied with the appropriate guidelines?

Yes

Reviewer 1 recommendation and comments

<https://doi.org/10.1099/acmi.0.000569.v1.4>

© 2023 Anonymous. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License.

Anonymous.

Date report received: 17 March 2023

Recommendation: Minor Amendment

Comments: Overall well written manuscript. These rare infections are important in patients with risk factors and timely diagnosis and treatment is necessary for good outcomes. Few minor suggestions to improve the manuscript 1. Case report a. Developed fever instead of 'a fever' b. Exacerbated on supine position instead of supination c. Can remove - As a part of evaluation CXR, USG and CT were done. Instead, can write CXR and USG findings. d. Can mention what all sputum and BAL investigations were done and negative (bacterial culture, fungal culture, TB, others) e. How was lung biopsy done ? Open lung biopsy or other method? f. Why was a repeat lung biopsy was done when patient was clinically improving ? g. Was the patient put on prophylaxis for nocardiosis after clinical recovery/cure? 2. Discussion a. Tacrolimus levels - units in ng/mL 3. Figure 1 - Legend A encircles the largest nodule - it should be consolidation

Please rate the quality of the presentation and structure of the manuscript

Good

To what extent are the conclusions supported by the data?

Strongly support

Do you have any concerns of possible image manipulation, plagiarism or any other unethical practices?

No

Is there a potential financial or other conflict of interest between yourself and the author(s)?

No

If this manuscript involves human and/or animal work, have the subjects been treated in an ethical manner and the authors complied with the appropriate guidelines?

Yes

SciScore report

<https://doi.org/10.1099/acmi.0.000569.v1.1>

© 2023 The Authors. This is an open-access article report distributed under the terms of the Creative Commons License.

iThenticate report

<https://doi.org/10.1099/acmi.0.000569.v1.2>

© 2023 The Authors. This is an open-access article report distributed under the terms of the Creative Commons License.