Nocardia isolation from clinical samples with the paraffin baiting technique

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Abstract

Background The genus *Nocardia* is a cause of infection in the lungs, skin, brain, cerebrospinal fluid, eyes, joints and kidneys. *Nocardia* isolation from polymicrobial specimens is difficult due to its slow growth. Several methods have been reported for *Nocardia* isolation from clinical samples. In the current study, we used three methods: paraffin baiting technique, paraffin agar, and conventional media for *Nocardia* isolation from various clinical specimens from Iranian patients.

Methods In this study, we examined 517 samples from various clinical specimens such as: sputum of patients with suspected tuberculosis, bronchoalveolar lavage, sputum of patients with cystic fibrosis, tracheal aspirate, cutaneous and subcutaneous abscesses, cerebrospinal fluid, dental abscess, mycetoma, wound, bone marrow biopsy, and gastric lavage. All collected specimens were cultured on carbon-free broth tubes (paraffin baiting technique), paraffin agar, Sabouraud dextrose agar, and Sabouraud dextrose agar with cycloheximide and were incubated at 35°C for one month.

Results Seven *Nocardia* spp. were isolated with paraffin baiting technique, compared with 5 positive results with the paraffin agar technique and 3 positive results with Sabouraud dextrose agar with and without cycloheximide. The prevalence of nocardial infections in our specimens was 5.28%.

Conclusion In the present study, the use of the paraffin baiting technique appeared to be more effective than other methods for *Nocardia* isolation from various clinical specimens.

Keywords *Nocardia*, paraffin baiting technique, paraffin agar, Sabouraud dextrose agar

Introduction

Nocardia spp. belong to a diverse group of bacteria known as aerobic actinomycetes, characterized as being Gram-positive rods with partially acid-fast, non-motile, filamentous branches; other properties include being catalase-positive and methenamine silver-positive.¹⁻³

Some members of the genus are opportunistic pathogens² that can be found around natural environments. This bacterium is not part of normal microbial flora in humans or in animals^{4, 5} and there is no report of person to person transmission.⁴ These microorganisms were first described by Edmond Nocard in 1888.^{1,5} *Nocardia* species are acquired via

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inhalation of aerosols or skin damage⁶ and can cause serious infections in different parts of the body especially in the lungs and skin.⁵

In recent years, the rate of nocardiosis has increased in patients with immune disorder diseases such as pemphigus disorder, Behçet's disease, malignancy and organ transplantation.⁷⁻⁹ Clinical diagnosis in nocardiosis is controversial and clinical signs are not specific for the bacteria. Recently, isolation and identification of *Nocardia* from clinical specimens has improved.^{10,11} Conventional approaches for detection of nocardial infection are isolation and identification of the bacterium by microbiological tests.^{11,12} *Nocardia* members are slow growing bacteria and their isolation from polymicrobial specimens such as sputum is difficult in the clinical microbiology laboratory¹³ therefore decontamination procedures are required for polymicrobial specimens, but these procedures may be toxic and hoarse.^{5,14} Paraffin baiting technique, paraffin agar, and media with or without anti-bacterial and anti-fungal agents^{13,15-18} were all used for isolation of *Nocardia* spp. The paraffin baiting technique was reported as being successful for the isolation of *Nocardia* from various clinical specimens especially polymicrobial samples such as sputum.^{13,17}

This study is unique for two reasons. The first aim of this study was efficacy and comparison of paraffin baiting technique with other methods such as conventional media including Sabouraud dextrose agar, Sabouraud dextrose agar with cycloheximide, and paraffin agar, to isolate *Nocardia* from various clinical specimens. The second aim was to estimate the prevalence of *Nocardia* infection in Iranian patients. There are few case reports of *Nocardia* infection and there is no comprehensive database of nocardiosis, therefore, it is essential to better assess the prevalence of infection with this bacteria.

Methods

Sample collection

Five hundred and seventeen various clinical specimens such as sputum of patients with suspected tuberculosis, sputum of patients with cystic fibrosis, bronchoalveolar lavage (BAL), cutaneous and subcutaneous abscesses, cerebrospinal fluid (CSF), dental abscess, mycetoma, wound, bone marrow biopsy, gastric lavage and tracheal aspirate were collected from February 28, 2011 to March 8, 2013 (Table 1). All clinical specimens except for CSF and bone marrow biopsy were homogenized and centrifuged at 10000 rpm for 10 minutes and the supernatant was discarded.

Culture on different media

The sediment of specimens was inoculated on Sabouraud dextrose agar, Sabouraud dextrose agar (Merck, Germany) with cycloheximide (Sigma-Aldrich, USA), paraffin agar (0.05 g FeSO₄, 0.05 g MgSO₄·7H₂O, 1 g K₂HPO₄, 5 g NH₄Cl, 0.05 g ZnSO₄, 1 g NH₄NO₃, 0.05 g MnSO₄, 3 g KH₂PO₄, 17 g Bacto Agar and 1 liter distilled water) and McClung's carbon-free broth tube (0.5 g MgSO₄·7H₂O, 2 mg ZnSO₄, 10 mg FeCl₃, 8 mg MnCl₂·4H₂O, 0.8 g K₂HPO₄, 2 g NaNO₃, 1 liter distilled water, pH 7.2) with placement of a paraffin coated glass rod. The pH was arranged with HCl (1N) and NaOH (1N). The tubes were incubated at 35°C for one month with daily control.^{13,16,18}

Cream to white-colored colonies appearing on the paraffin-coated glass rod were selected and subcultured on nutrient agar, and were then purified and stained with Gram, modified Kinyoun acid-fast (partially acid-fast) and Kinyoun acid-fast (cold procedure). The difference between modified Kinyoun acid-fast and Kinyoun acid-fast resides in decolorization.^{5,19} Some of the phenotypic tests and growth to lysozyme broth were used for identification of the genus *Nocardia*.^{20,21}

Results

In McClung's method, cream to white-colored colonies appeared on the paraffin-coated glass rod. Colonies were cultured on nutrient agar and were purified (Figure 1). Smears performed from

the colonies revealed Gram-positive microorganisms; partially acid-fast stains (modified Kinyoun acid-fast) were also positive (Figure 2) but Kinyoun stains were negative in all isolates. Colony morphology was similar to that of the genus *Nocardia* and all clinical isolates were grown in lysozyme broth medium (Figure 3). Seven strains were isolated with paraffin baiting technique as compared with Sabouraud dextrose agar, Sabouraud dextrose agar with cycloheximide and paraffin agar (Table 1). The prevalence of nocardiosis is shown in Table 2. In our study, we also isolated one *Nocardia* spp. from a cutaneous abscess in a patient with pemphigus disorder.

Discussion

Nowadays nocardiosis occurs more frequently in patients with weak immune systems and the most common form is pulmonary nocardiosis, displaying symptoms that mimic tuberculosis, but requiring a different type of treatment.^{1,7,9} In scientific literature, some documents recommend the use of paraffin baiting technique for isolation of *Nocardia* spp. from polymicrobial specimens such as sputum, because *Nocardia* utilizes paraffin wax as the sole carbon source.^{22,23} In a study by Mishra

Table 1. Comparative results of paraffin baiting technique and conventional techniques of direct culture on Sabouraud dextrose agar (SDA), Sabouraud dextrose agar with cycloheximide and paraffin agar (PA) in the isolation of *Nocardia* spp. from various clinical specimens

Clinical samples	Number of cases	Paraffin baiting method	PA	SDA	SDA+ cycloheximide	p-value
Sputum of patients with suspected tuberculosis	238	4	2	1	1	p>0.05
Sputum of patients with cystic fibrosis	53	0	0	0	0	
Bronchoalveolar lavage	143	2	2	1	1	
Cutaneous abscesses	45	1	1	1	1	
CSF	1	0	0	0	0	
Mycetoma	2	0	0	0	0	
Dental abscess	1	0	0	0	0	
Tracheal aspirate	31	0	0	0	0	
Wound	1	0	0	0	0	
Bone marrow biopsy	1	0	0	0	0	
Gastric lavage	1	0	0	0	0	
Total	517	7	5	3	3	

Table 2. Prevalence of nocardiosis in clinical samples

Clinical samples	Sputum (n=238)	BAL (n=143)	Cutaneous abscess (n=45)	Total samples (n=517)
Positive	(4) 1.68%	(2) 1.4%	(1) 2.2%	(7) 5.28%

Figure 1. *Nocardia* grown on nutrient agar medium

Figure 2. Partially acid-fast staining of *Nocardia*

Figure 3. Growth of *Nocardia* in lysozyme broth

and colleagues in 1969, they investigated 555 clinical specimens such as sputum, BAL, and gastric lavage by paraffin baiting technique and found results of 10, 1 and 1 respectively for *Nocardia* spp.¹⁶ Singh et al. surveyed 1510 sputum specimens and their results showed that paraffin baiting method had higher efficacy compared to Sabouraud

dextrose agar.¹⁸ In another study by Venugopal et al. 350 sputum, BAL, pleural fluid, pus and biopsy specimens were examined and 15 isolates were recovered.²⁴ In this study paraffin baiting method was more suitable than other methods for *Nocardia* isolation. Eshraghi et al. in 2001 evaluated the efficiency of Sabouraud dextrose agar and paraffin agar for nocardial isolation from 142 sputum specimens and just one specimen was positive but in our study, 4 specimens (of 291 sputum samples) were positive.²⁵ The results show that nocardiosis appears to be increasing in Iranian patients and this should be investigated further in future studies. In the present study, noticeably, no statistical differences among methods were observed. This is mainly because statistical tests severely focus on sample size, and don't analyze things from microbiological perspective. However, in microbiology, based on the observation that there is a certain difference among these methods, the paraffin baiting technique can be considered the best method. Biochemical tests and molecular methods such as polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and PCR-sequencing are also important for accurate identification of genus and species of *Nocardia*.^{5,26,27}

Conclusion

In comparison to various media, paraffin baiting technique appeared to be effective and specific for *Nocardia* spp. isolation from various clinical specimens, especially polymicrobial samples.

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Conflicts of interest

All authors – none to declare

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