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Necessity of Studying the Methods for Isolation of *Nocardia* from Environmental and Clinical Samples

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Dear Editor,

Nocardia is a genus of gram-positive, aerobic, slow growth, and partial acid fast bacteria. It is free-living species (saprophytic) existing in environmental resources such as water, soil, dust, animal waste and rotten plants. Therefore, the patients with immunodeficiency have a high chance of getting infections with these bacteria. According to available evidence, *Nocardia* species enter the human body through dust, ventilation systems and traumatic inoculations and cause Noccardial infections in patients with immunodeficiency and the healthy individuals [1, 2].

Nocardial infections often occur as Nocardial pneumonia infections, brain abscesses, cutaneous, lymphatic, and lympho-cutaneous infections. The isolation of *Nocardia* species is of high significance due to the clinical importance and the production of secondary metabolites, especially antibiotics [1-4]. However, it should be noted that it is difficult to isolate *Nocardia* species because of the complexities involved in isolating and identifying this group of bacteria. The clinical manifestations and radiological evidence of nocardial pulmonary infections are nonspecific and the diagnosis of nocardiosis based on the clinical findings is impossible. Also, the *Nocardia* species are slowly growing, thus, other fast-growing bacteria prevent the isolation of these bacteria. Also diagnosis of nocardiosis based on direct examination and conventional cultures, is difficult, long, time-consuming and given to time-consuming conventional tests, nocardiosis is sometimes diagnosed after the dissemination of the strain to other organs or after the patient has died; so we need for new methods for identification of *Nocardia* infections. The molecular methods such as 16S rRNA-restriction fragment length polymorphism (16S rRNA-RFLP), *hsp65*-RFLP, direct sequencing of 16S rRNA and *hsp65* was eliminates these problems by the specific amplification of *Nocardia* genome even after the beginning of chemotherapy [3, 5].

Different confirmed scientific methods have been introduced to isolate these bacteria from clinical and environmental samples, including: Paraffin agar, Paraffin baiting technique, Humic acid vitamin B agar, Selective BCYE (Buffered charchol-yeast exctract), Slip-buried method, sucrose-gradient centrifugation, gelatin agar (GA), Blood agar and urea agar [3]. Among the methods mentioned above, the Paraffin baiting technique is one of the most important methods for the isolation of *Nocardia* species from clinical and environmental samples which is widely used. *Nocardia* species having paraffin degrading enzymes are able to use this compound as the only source of carbon and energy. Therefore, one can isolate *Nocardia* species from polymicrobial samples such as soil or sputum easily using an organic-free medium (for example McClung medium) and adding paraffin rods to it. Although it has been shown that some Pseudomonas species can also use paraffin, this method has been used in several studies on the isolation of *Nocardia* species from clinical and soil samples and has the advantage of isolating more *Nocardia* species compared to other methods [6-9]. According to Hayakawa et al. other genera of Streptomyces, Micromonospora, Microbispora, Streptosporangium, Dactylosporangium, Microtetraspora and Thermomonospora are also isolated by Humic acid-vitamin B agar technique in addition to *Nocardia*. Therefore, HV agar is not a specific environment for *Nocardia* species [3]. Eshraqi et al. found that paraffin agar is not a specific method for the isolation of *Nocardia* species due to contamination with other microorganisms [3]. The Slip-buried technique is not also a specific method for the isolation of *Nocardia* species due to the use of Streptomycin and Chloramphenicol antibiotics and the sensitivity of some *Nocardia* species to these antibiotics [10]. It is difficult and time consuming to isolate *Nocardia* species using phenotypic methods and it is suggested to use molecular methods such as hybridization or examination of 16S rRNA and *hsp65* genes in urgent cases and need for immediate diagnosis of *Nocardia* infections. Nevertheless, it should be noted that phenotypic methods are known as gold stan-

dard, and molecular methods are complementary to these methods. Also, molecular techniques' equipment are expensive and require trained technicians. Thus, it is not possible to use molecular techniques in all medical diagnostic laboratories in developing countries [4, 11].

In summary, isolation and identification of *Nocardia* species are important due to their clinical and industrial application and studies on the identification of new species of *Nocardia*. According to the research, it can be stated that the Paraffin baiting technique is one of the most reliable methods for the isolation of *Nocardia* species from clinical and soil samples. In addition, it is likely in clinical cases that *Nocardia* attacks other organs of the patient body and causes the patient' death prior to phenotypic identification. Therefore, in such cases, molecular methods can be used.

Footnote

Conflict of Interest: There is no conflict of interest.

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