Genetic diversity and antimicrobial susceptibility of *Nocardia* species among patients with nocardiosis

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The aim of this multicenter study was to determine the genetic diversity and antibiotic susceptibility of clinically isolated *Nocardia* species. One hundred twenty-seven patients with nocardiosis were randomly selected from 5 provinces of Iran. Molecular diagnosis of *Nocardia* species was performed using multilocus sequence analysis of gyrase B of the β subunit of DNA topoisomerase (*gyrB*), and 16S rRNA and subunit A of SecA preproteintranslocase (*secA1*). Antimicrobial susceptibility testing was performed following the Clinical and Laboratory Standards Institute recommendations. Thirty-five *N. cyriacigeorgica*, 30 *N. asteroides*, 26 *N. farcinica*, 12 *N. otitidiscaviarum*, and 10 *N. abscessus* cultures were studied. All isolates were susceptible to linezolid. All isolates of *N. cyriacigeorgica*, *N. asteroides*, *N. abscessus*, and *N. otitidiscaviarum* were susceptible to trimethoprim-sulfamethoxazole, while 8% of *N. farcinica* isolates were resistant to this drug. All *N. otitidiscaviarum* isolates were highly resistant to imipenem, but *N. cyriacigeorgica*, *N. asteroides*, *N. farcinica*, and *N. abscessus* were only moderate resistant. The susceptibility patterns vary with different species of *Nocardia*. Resistance to trimethoprim-sulfamethoxazole in Iran is low and this drug should be first line therapy, unless drug susceptibility testing shows resistance. Linezolid also covers *Nocardia* well and could be a second line agent.

*Nocardia* can be found worldwide as a saprophytic pathogen in water, soil, decaying fecal deposits from animals and other ecological niches. Only a small proportion of the currently described *Nocardia* species are known to be human pathogens that affect both immunosuppressed and immunocompetent patients. Nocardial infections range from minor cutaneous lesions to severe pulmonary or central nervous system disease. The incidence rates of *Nocardia* species isolation from clinical samples have been increasing worldwide in the recent decades. The reason for this increase could be related to advances in culturing and improved molecular methods as well as progress in oncology, rheumatology, and transplant medicine. Drug susceptibility testing of *Nocardia* isolates is recommended as a guide to therapy for cases of severe or disseminated infection, refractory cases, and those who are intolerant to treatment with sulfonamides. However, there is limited information about the distribution of the different *Nocardia* species and drug susceptibility of *Nocardia* worldwide including the Middle East. The aim of this study was to determine genetic diversity and drug susceptibility of clinical isolates of *Nocardia* from Iran.
Material and methods

Organisms. One hundred twenty-seven clinical isolates of *Nocardia* from different major cities of Iran were studied between 2009 and 2015; 22 were from Khosestan (southwest Iran), 47 from Tehran (central Iran), 21 from Mazandaran (northern western Iran), and 10 from Kermanshah (northeast Iran) (Fig. 1). This study approved by Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (AJUMS), Ahvaz, Iran. Demographic, clinical, and microbiologic data were collected from patients' medical records who signed the informed written consent. Isolates were sent to the Infectious and Tropical Diseases Research Center (AJUMS) for identification and subsequently antimicrobial susceptibility determination. All experimental protocols including sample collection and laboratory methods were approved by scientific committee of Health Research Institute (AJUMS).

A portion of the isolates (32 specimens) were identified at the species level by multilocus sequence analysis (MLSA) of 16S rRNA, gyrase B of the β subunit of DNA topoisomerase (gyrB) and subunit A of SecA preprotein translocase (secA1) as previously described by McTaggart and colleagues to find out the reliability of each marker for identification. The remaining isolates were identified to species level by 16S rRNA analysis because of its acceptable discriminatory power. The 16S rRNA gene was amplified using 27F primer (5′–AGAGTTTGATCCTGGCTCAG–3′) and 1525R (5′–AAGGAGGTGWTCCARCC–3′) and then was sequenced. The sequences were aligned and trimmed in BioNumerics (version 6.0.1) software (Applied Maths, Austin, TX) and were identified to species level. A representative 16S rRNA gene sequence from each of species was deposited in Genbank with KT003507-KT003513 accession numbers.

Broth microdilution testing. The drugs amikacin, amoxicillin-clavulanate, cefepime, cefotaxime, ceftriaxone, ciprofloxacin, clarithromycin, doxycycline, gentamicin, imipenem, linezolid, minocycline, moxifloxacin, tobramycin, trimethoprim-sulfamethoxazole (TMP-SMZ), and vancomycin were selected by testing based on National Committee for Clinical Laboratory Standards (NCCLS) recommendations. Microtiter plates were prepared in-house, using standard twofold dilution of all antimicrobials except ampicillin and amoxicillin-clavulanate in cation-adjusted Mueller-Hinton broth. The plates were stored at ~70°C and were thawed at room temperature immediately before use. The appropriate dilution of amoxicillin-clavulanate was freshly prepared immediately before use, then aliquoted, and placed in designated microtiter wells. Ten microliters of an inoculum with a turbidity equivalent to that of a 0.5 to 1.0 McFarland standard was dispensed into each well to give a final concentration of 10^4 to 10^5 CFU/mL. The microtiter plates were incubated aerobically at 35°C and were read after 3 days. Growth was examined daily by visual inspection. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the drug that inhibited visible growth. MICs at which 50% (MIC50s) and 90% (MIC90s)
of isolates are inhibited were determined. MIC50% and MIC90% were selected to provide an interpreta-
tion of the clinical significance of concentrations of an antimicrobial that inhibit the growth of an organ-
ism or kill it in laboratory systems (in vitro) and for defining the starting point for larger preclinical
evaluations of novel antimicrobial agents. For TMP-SMZ, the MIC was the 80% inhibition endpoint
growth compared to the control. Susceptible and resistant breakpoints were defined according to the
NCCLS recommendations. Quality control of the MICs was performed by the testing of NCCLS recom-
mended reference strains, including Enterococcus faecalis ATCC 29212, Nocardia abscessus DSM 44432,
Pseudomonas aeruginosa ATCC 27853, and Staphylococcus aureus ATCC 29213.
The control strains were
obtained from Iranian Biological Resource Center (IBRC), Tehran, Iran.

Results
Out of 127 patients with nocardiosis 69 (54%) were females. The mean age was 47.6 (SD = 21) years.
Table 1 shows the demographic information of study population. Almost half of the patients had at
least one significant underlying condition, such as solid organ transplantation (11 patients, 8.7%), solid
or hematologic malignancy (10 patients, 8%), HIV (13 patients, 10%), and receiving corticosteroids
for rheumatologic disorders (9 patients, 7%). No known immunodeficiency was found in 56 (44%)
patients. The most common symptoms were fever (52%) and cough (47%) in patients with pulmonary
disease. Cavitary lesion was found on chest radiography in 18 (30%) persons with pulmonary disease.
Pleural effusions occurred in 8 (14%). Lungs were the primary organ involved in 64 (50%) patients.
Extrapulmonary nocardiosis included skin and soft tissue (31 persons, 24%) and central nervous system
disease (brain abscess) (12 persons, 9%), and disseminated disease (18 persons, 14%). Bronchoalveolar
fluid was the most common source of Nocardia isolation (60%). Extrapulmonary nocardiosis occurred
more often in younger individuals (mean age 38.6 versus 55.8 in pulmonary group, p < 0.0001) and those
who were taking corticosteroids for rheumatologic disorders (8, 13% vs. 1, 2% in pulmonary group,
p = 0.011). Out of 127 clinical isolates, 31 (24%) were N. asteroides, 25 (20%) were N. cyriacigeorgica,
26(21%) were N. farricina,12 (9%) were N. otitidiscavarum,19 (15%) were N. abscessus, 6(5%) were N.
wallacei, 3 (2%) were N. carnea, 2 (2%) were N.nova, and one each were from N. kruczka,G. veterana,
and N. arthritidis. Some of these data were published elsewhere.

Table 2 shows that the Nocardia species most commonly
isolated from human infections were N. asteroides, N. farcinica genotype I, and N. cyriacigeorgica genotype I.
Isolates N 6, N 7, N 35, N 48, N 49, N 50, N 66 and N 67 were identified N. asteroides genotype II;
N 9, N 10, N 21 and N 32 were grouped as N. asteroides genotype III, while N 18, N 19, N 20, N61,
N 62, N 71, N 78 were clustered as N. asteroides genotype I.
Drug susceptibility testing. Table 3 presents the MICs at which 50% (MIC50s) and 90% (MIC90s) of isolates are inhibited and the range of MICs for all Nocardia isolates. All Nocardia isolates were resistance to vancomycin.

**N. asteroides.** Among the 31 isolates of N. asteroides, all were susceptible to TMP-SMZ and linezolid. Amoxicillin-clavulanic acid, cefepime, ceftriaxone, ciprofloxacin, imipenem, moxifloxacin, and tobramycin had moderate activity, while clarithromycin had poor activity against the clinical isolates of N. asteroides. The MIC90 for both linezolid and TMP-SMZ was 1 (μg/ml), but, for ceftriaxone this value was 128 (μg/ml).

**N. farcinica.** All 26 isolates of N. farcinica were susceptible to amikacin and linezolid, and all were resistant to ceftriaxone, doxycycline, gentamicin, minocycline, and tobramycin. Two (8%) of 26 isolates were resistant to TMP-SMZ. For amikacin and linezolid the lowest concentration of MIC90 (1 μg/ml)
| Species (number of isolates)/antibiotics | MIC (μg/ml) | Number (%) of isolates |
|----------------------------------------|-------------|------------------------|
|                                        | 50% | 90% | Susceptible | Intermediate | Resistant |
| N. cyriacigeorgica (25)                 |     |     |             |              |         |
| Amikacin                               | 0.125 | 1   | 25(100)     |              | 0 (0)    |
| Amoxicillin-clavulanic acid            | 8    | 64  | 0(0)        | 5(20)        | 20(80)   |
| Ceftriaxone                            | 4    | 64  | 20(80)      | 2(8)         | 3(12)    |
| Ciprofloxacin                          | 4    | 32  | 0(0)        | 7(28)        | 18(72)   |
| Clarithromycin                         | 2    | 8   | 8(32)       | 7(28)        | 10(40)   |
| Ceftazidime                             | 0.125 | 0.5 | 25(100)     | 0(0)         | 0(0)     |
| Cefotaxime                              | 4    | 64  | 20(80)      | 1(4)         | 4(16)    |
| Gentamicin                              | 1    | 2   | 25(100)     | 0(0)         | 0(0)     |
| Doxycycline                             | 0.25 | 16  | 18(72)      | 3(12)        | 4(16)    |
| Imipenem                                | 1    | 64  | 15(60)      | 2(8)         | 8(32)    |
| Linzolid                                | 0.125 | 0.5 | 25(100)     |              |         |
| Minocycline                             | 8    | 32  | 0(0)        | 3(8)         | 22(88)   |
| Moxifloxacin                            | 32   | 64  | 0(0)        | 0(0)         | 25(100)  |
| Tobramycin                              | 0.125 | 0.5 | 25(100)     | 0(0)         | 0(0)     |
| TMP-SMZ                                 | 0.125 | 0.5 | 25(100)     |              |         |
| N. asteroidis(31)                       |     |     |             |              |         |
| Amikacin                                | 2    | 16  | 24(77)      |              | 7 (23)   |
| Amoxicillin-clavulanic acid            | 16   | 32  | 17(55)      | 3(10)        | 11(35)   |
| Ceftriaxone                            | 4    | 128 | 17(55)      | 3(10)        | 11(35)   |
| Ciprofloxacin                          | 0.125 | 8  | 17(55)      | 5(16)        | 9(29)    |
| Clarithromycin                         | 16   | 32  | 2(6)        | 3(10)        | 26(84)   |
| Ceftazidime                             | 16   | 64  | 12(39)      | 5(16)        | 14(45)   |
| Cefotaxime                              | 32   | 64  | 10(32)      | 9 (29)       | 12(39)   |
| Gentamicin                              | 0.125 | 4  | 28(90)     | 0(0)         | 3(10)    |
| Doxycycline                             | 0.125 | 32 | 16(52)      | 7(23)        | 8(25)    |
| Imipenem                                | 1    | 32  | 28(90)      | 0(0)         | 3(10)    |
| Linzolid                                | 0.125 | 1  | 31(100)     |              |         |
| Minocycline                             | 8    | 32  | 8(25)       | 10(32)       | 13(43)   |
| Moxifloxacin                            | 8    | 16  | 12(39)      | 8(25)        | 11(35)   |
| Tobramycin                              | 4    | 16  | 19(61)      | 5(16)        | 7(23)    |
| TMP-SMZ                                 | 0.5  | 1   | 31(100)     |              | 0 (0)    |
| N. farcinica (26)                       |     |     |             |              |         |
| Amikacin                                | 0.125 | 1  | 26(100)     |              | 0 (0)    |
| Amoxicillin-clavulanic acid            | 2    | 32  | 16 (61)     | 2(8)         | 8(31)    |
| Ceftriaxone                            | 128  | 256 | 0 (0)       | 0(0)         | 26(100)  |
| Ciprofloxacin                          | 0.125 | 2  | 10(38)      | 5(20)        | 11(42)   |
| Clarithromycin                         | 8    | 32  | 0(0)        | 4(15)        | 22(85)   |
| Ceftazidime                             | 32   | 64  | 0(0)        | 4(15)        | 22(85)   |
| Cefotaxime                              | 32   | 64  | 0(0)        | 0(0)         | 26(100)  |
| Gentamicin                              | 32   | 128 | 0(0)        | 0(0)         | 26(100)  |
| Doxycycline                             | 16   | 32  | 0(0)        | 0(0)         | 26(100)  |
| Imipenem                                | 1    | 32  | 15(58)      | 3(11)        | 8 (31)   |
| Linzolid                                | 0.125 | 1  | 26(100)     |              |         |
| Minocycline                             | 8    | 32  | 0(0)        | 0(0)         | 26(100)  |
| Moxifloxacin                            | 4    | 16  | 10(38)      | 3(11)        | 13(51)   |
| Tobramycin                              | 16   | 32  | 0(0)        | 0(0)         | 26(100)  |

Continued
was detected. Ceftriaxone had the highest concentration of MIC90 value between all tested antibiotics (256 μg/ml).

**N. cyriacigeorgica.** The 25 *N. cyriacigeorgica* clinical isolates were susceptible to amikacin, cefepime, gentamicin, linezolid, tobramycin, and TMP-SMZ. *N. cyriacigeorgica* generally had good sensitivity to cefotaxime, ceftriaxone, clarithromycin, doxycycline, imipenem, and minocycline, but poor sensitivity to amoxicillin-clavulanic acid, ciprofloxacin, and minocycline. All isolates were resistant to moxifloxacin. The MIC90 for cefepime, linezolid, tobramycin and TMP-SMZ was 0.5 μg/ml and for amoxicillin-clavulanic acid, ceftriaxone, cefotaxime, imipenem and moxifloxacin was 64 (μg/ml).

| Species (number of isolates)/antibiotics | MIC (μg/ml) | Number (%) of isolates |
|----------------------------------------|-------------|------------------------|
|                                         | 50%  | 90%  | Susceptible | Intermediate | Resistant |
| TMP-SMZ                                | 0.5  | 8    | 24(92)      | —            | 2(8)      |

**N. otitidiscaviarum (12)**

| Antibiotics                                | MIC (μg/ml) | Number (%) of isolates |
|--------------------------------------------|-------------|------------------------|
| Amikacin                                   | 0.125       | 2  | 12(100) | — | 0(0) |
| Amoxicillin-clavulanic acid                | 32  | 64 | 0(0)   | 0(0) | 12(100) |
| Ceftriaxone                                | 64  | 256 | 0(0)  | 2(17) | 10(83) |
| Ciprofloxacin<sup>a</sup>                  | 4   | 8  | 2(16)  | 3(25) | 7(58) |
| Clarithromycin<sup>b</sup>                 | 4   | 16 | 4(33)  | 1(8)  | 7(58) |
| Cefepime                                   | 16  | 32 | 4(33)  | 4(33) | 4(33) |
| Cefotaxime                                 | 64  | 128 | 2(17) | 1(8)  | 9(75) |
| Gentamicin                                 | 2   | 32 | 6(50)  | 0(0)  | 6(50) |
| Doxycycline                                | 8   | 32 | 1(8)   | 1(8)  | 10(83) |
| Imipenem                                   | 16  | 64 | 0(0)   | 0(0)  | 12(100) |
| Linezolid<sup>c</sup>                      | 0.125 | 1  | 12(100) | — | — |
| Minocycline                                | 8   | 32 | 1(8)   | 1(8)  | 10(83) |
| Moxifloxacin                               | 4   | 16 | 3(25)  | 3(25) | 6(50) |
| Tobramycin                                 | 0.5  | 1  | 12(100) | 0(0)  | 0(0) |
| TMP-SMZ                                    | 0.125 | 0.5 | 12(100) | — | 0(0) |

**N. abscessus (19)**

| Antibiotics                                | MIC (μg/ml) | Number (%) of isolates |
|--------------------------------------------|-------------|------------------------|
| Amikacin                                   | 16  | 32 | 7(37)   | — | 12(63) |
| Amoxicillin-clavulanic acid                | 4   | 64 | 8(42)   | 2(11) | 9(47) |
| Ceftriaxone                                | 0.5  | 1  | 19(100) | 0(0)  | 0(0) |
| Ciprofloxacin<sup>a</sup>                  | 4   | 16 | 0(0)   | 0(0)  | 19(100) |
| Clarithromycin<sup>b</sup>                 | 4   | 16 | 6(31)  | 3(16) | 10(53) |
| Cefepime                                   | 8   | 32 | 10(53) | 3(16) | 6(31) |
| Cefotaxime                                 | 8   | 64 | 10(53) | 3(16) | 6(31) |
| Gentamicin                                 | 0.5  | 1  | 19(100) | 0(0)  | 0(0) |
| Doxycycline                                | 1   | 8  | 4(21)  | 3(16) | 12(63) |
| Imipenem                                   | 8   | 32 | 4(21)  | 2(11) | 13(68) |
| Linezolid<sup>c</sup>                      | 0.125 | 1  | 19(100) | — | — |
| Minocycline                                | 4   | 16 | 5(26)  | 2(11) | 12(63) |
| Moxifloxacin                               | 4   | 16 | 0(0)   | 0(0)  | 19(100) |
| Tobramycin                                 | 0.5  | 1  | 19(100) | 0(0)  | 0(0) |
| TMP-SMZ                                    | 0.125 | 0.5 | 19(100) | — | 0(0) |

Table 3. Drug susceptibility testing results for clinical isolates of *Nocardia*. <sup>a</sup>Ciprofloxacin may be used as a class representative for the older fluoroquinolones: ciprofloxacin, ofloxacin, and levofloxacin.<br><sup>b</sup>Class representative for newer macrolides. <sup>c</sup>Proposed breakpoint with linezolid MIC values >8μg/mL for *Nocardia* isolates have been adapted from reference<sup>37</sup>. Breakpoints are arbitrary since there are currently no NCCLS interpretive criteria.
**N. abscessus.** All 19 isolates of *N. abscessus* were susceptible to ceftriaxone, gentamicin, linezolid, tobramycin, and TMP-SMZ. Cefepime, cefotaxime, doxycycline, imipenem, and minocycline showed good activity against *N. abscessus* isolates. Amikacin, amoxicillin-clavulanic acid, and clarithromycin had low activity against the clinical isolates of *N. abscessus*, and ciprofloxacin, moxifloxacin had no activity against these isolates. Ceftriaxone, gentamicin, linezolid and tobramycin had MIC90 (1 μg/ml). The MIC90 for amoxicillin-clavulanic acid and cefotaxime was 64 μg/ml and for amikacin, cefepime and imipenem was 32 μg/ml.

**N. otitidiscaviarum.** All 12 isolates of *N. otitidiscaviarum* were susceptible to amikacin, linezolid, tobramycin, and TMP-SMZ, whereas there was no activity to ceftriaxone, doxycycline, and minocycline, and all isolates were resistant to amoxicillin-clavulanic acid, imipenem. The MIC90 for amikacin, linezolid and TMP-SMZ was 0.125 μg/ml.

**N. wallacei.** All 6 isolates were resistance to amikacin, clarithromycin, imipenem, moxifloxacin were susceptible to ceftriaxone, cefepime, cefotaxime, gentamicin, linezolid, tobramycin and TMP-SMZ. Amoxicillin-clavulanic acid, doxycycline and minocycline demonstrated poor activity against the isolates.

**N. carneae.** All 3 isolates of *N. carneae* were resistance to amikacin, amoxicillin-clavulanic acid, ceftriaxone, ciprofloxacin, clarithromycin, imipenem, moxifloxacin were susceptible to cefepime, cefotaxime, gentamicin, linezolid, tobramycin and TMP-SMZ. Poor activity was recorded for doxycycline and minocycline.

One isolate from each species of *N. arthritidis, N. kruczakiae, N. nova* and *N. veteran* were studied for drug susceptibility tests. All of them were susceptible to amikacin, amoxicillin-clavulanic acid, ceftriaxone, ciprofloxacin, clarithromycin, imipenem, linezolid, tobramycin and TMP-SMZ.

**Discussion**

*N. asteroides* was the most frequently recovered species in our study. It was followed by *N. farcinica* and *N. cyriacigeorgica*. This pattern was different between individuals with pulmonary and extrapulmonary nocardiosis, with *N. cyriacigeorgica* being the most common in extrapulmonary disease. Our study found that extrapulmonary nocardiosis occurs more commonly in younger persons (mean age 38) compared to pulmonary nocardiosis (mean age 56) and in those with rheumatologic disorders taking corticosteroids.

Although cases reports have shown *N. cyriacigeorgica*4, *N. asteroides* complex15 and *N. nova* complex16 in Iran, but to our knowledge, there is no report of drug susceptibility on clinical isolates of *Nocardia* from Iran as well as the Middle Eastern countries.

The lungs are the most common organ that *Nocardia* infects (up to 70%), with *N. asteroides* complex the species most often isolated from this site17. Yamagata and colleagues reported that patients with rheumatologic disorders who took corticosteroid were at higher risk of extrapulmonary nocardiosis6. Our study confirmed the higher incidence extrapulmonary nocardiosis in those taking corticosteroids before the *Nocardia* infection. This information may serve as a warning to clinicians about the risk of corticosteroids and disseminated nocardial infection.

*Nocardia* species cause a wide variety of diseases and have variable drug susceptibility profiles. Since the 1940s, the sulfonamides have been the treatment of choice for nocardiosis1–18. Later, the combination of trimethoprim with sulfamethoxazole became the most commonly recommended treatment for these infections1. Other therapies including amikacin, a combination of amikacin and a beta-lactam such as ceftriaxone or imipenem, and a combination with linezolid have also been suggested for therapy of patients with serious disease1,19.

Susceptibility testing of *Nocardia* isolates to the antibiotics showed that *N. cyriacigeorgica* isolates were generally sensitive to our selected antibiotics. All were susceptible to amikacin, cefepime, gentamicin, linezolid, tobramycin, and TMP-SMZ; and the majority was somewhat less susceptible to cefotaxime, ceftriaxone, doxycycline, imipenem, and minocycline. These findings are consistent with those reported by Glupczynski and colleagues20. Further, Larruskin and colleagues noted that *N. cyriacigeorgica* isolates from Spain were susceptible to amikacin, gentamicin, linezolid, tobramycin, and TMP-SMZ21.

Schlaberg and colleagues from the United States reported that *N. cyriacigeorgica* isolates were susceptible to amikacin, linezolid, tobramycin, and TMP-SMZ, and were resistant to amoxicillin-clavulanic acid, ciprofloxacin, clarithromycin, minocycline, and moxifloxacin22. However, our isolates were highly resistant only to amoxicillin-clavulanic acid, ciprofloxacin, and moxifloxacin. Ceftriaxone, imipenem, linezolid, and TMP-SMZ were reported as the most effective antimicrobial agents against *N. cyriacigeorgica* isolates in Taiwan23, which agrees with our results.

Among the 31 isolates of *N. asteroides*, linezolid and TMP-SMZ were active against all isolates while moderate susceptibility was detected for imipenem, amoxicillin-clavulanic acid, cefepime, ceftriaxone, ciprofloxacin, moxifloxacin, and tobramycin. Clarithromycin had poor activity against clinical isolates of *N. asteroides* in our study. In the preliminary evaluation of antimicrobial agents against *N. asteroides* isolates in 1984, the beta-lactams including third-generation cephalosporins were generally reported ineffective, whereas minocycline, doxycycline, and sulfamethoxazole were recommended for therapy24. Four years later, Wallace and colleagues showed that the most active parenteral agents against *N. asteroides* were amikacin, cefotaxime, ceftriaxone, imipenem, minocycline, and sulfonamides25. Although Schlaberg
and colleagues found that all N. asteroides isolates were susceptible to amikacin, imipenem, linezolid, tobramycin, and TMP-SMZ, we found less susceptibility among N. asteroides isolates in our study.

N. farcinica is more likely to have multidrug resistance and high level resistance to imipenem, ceftriaxone, clarithromycin, tobramycin, and moxifloxacin. Although TMP-SMZ has been the drug of choice for the treatment of nocardiosis, we found 8% (2 isolates) of N. farcinica were TMP-SMZ resistant. Larruskain and colleagues in Spain found 16.1% and Uhde and colleagues found 42% from the United States, and Tremblay and colleagues also reported 42% TMP-SMZ resistant strains from Canada. Another study from Spain, reported that 9 of 19N. farcinica isolates (47%) were TMP-SMZ resistant. Furthermore, Lai and colleagues from Taiwan reported a low incidence (9%) similar to ours, and another report from the United States also found only 2% TMP-SMZ resistance and sulfonamide and TMP-SMZ resistance was not seen in South Africa. The similarity between the 2 North American countries and divergence in Europe and Iran suggests there may be geographical differences in N. farcinica drug sensitivity with unknown reasons. We speculate that the difference in drug susceptibility to TMP-SMZ could be related to differences in laboratory methodology and interpretation criteria. More recently, Valdezate and colleagues reported association of high-level sulfonamide resistance and the presence of plasmid-borne integrins carrying sul genes (sul1 and sul2) in SXT-resistant Nocardia strains. These type of integrins, and the corresponding plasmids, are commonly detected in bacteria living in different ecological niches.

In our study, resistance to β-Lactams antibiotics were detected among the isolates, which might be related to a mutational change affecting the inhibitor and active site(s) in the beta-lactamase. Our Nocardia isolates showed moderate resistance to quinolones. Valdezate and colleagues could not find plasmid-mediated quinolone resistance genes (qnrA, qnrB, qnrC, and qnr) or the gene for the aminoglycoside acetyltransferase for modify ciprofloxacin or efflux pump qepA and or nucleotide changes observed in gyrA. Further, study considering the resistance mechanisms and how antibiotic resistance spreading among Nocardia strains are required.

All N. otitidiscaviarum species were susceptible to TMP-SMZ. Our data was in agreement with those reported by others. In contrast with our data, moderate resistance to TMP-SMZ (32%) among N. otitidiscaviarum was reported by Uhde and colleagues. N. abscessus were susceptible to ceftriaxone, gentamicin, linezolid, tobramycin, and TMP-SMZ in our study. The same susceptibility profile of N. abscessus was reported before.

Linezolid, a relatively new class of antibiotics, showed extraordinary in vitro activity against all of the major clinically significant species of Nocardia. Our findings are in agreement with reports from different parts of world that clearly demonstrate that linezolid is an effective alternative for the treatment of nocardiosis.

In conclusion, N. asteroides was the most common species isolated from pulmonary nocardiosis and N. cyriacigeorgica was the most frequently recovered species from extrapulmonary nocardial infections. Clinical isolates of Nocardia species in our study had varied drug susceptibility patterns, which were similar to what have been reported from other geographic area, with some exceptions. Importantly, TMP-SMZ resistance was low in the current study. Based on this information, we feel confident recommending TMP-SMZ as the first choice for the treatment of nocardiosis in Iran. Linezolid broadly covers Nocardia and would be a second choice, although the costs are considerably greater. We strongly recommend that drug sensitivity testing is helpful in all patients with serious disease.

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**Acknowledgements**

We appreciate the financial support of Ahvaz Jundishapur University of Medical Science, Ahvaz, Iran.

**Author Contributions**

Conception, hypotheses delineation, and design of the study: M.M., A.H.S. and P.H. The laboratory tests, data analysis and interpretation of such information: M.M., A.H.S., PH., S.Z.B., M.H. and M.M.F. Writing the article or substantive involvement in its revision before submission: M.M., M.M.B., P.H. and D.S.

**Additional Information**

**Competing financial interests** Dr. Mehdi Mirsaedi who is a Federal Employee at Miami VA Medical Center.

**How to cite this article:** Hashemi-Shahraki, A. et al. Genetic diversity and antimicrobial susceptibility of Nocardia species among patients with nocardiosis. *Sci. Rep.* **5**, 17862; doi: 10.1038/srep17862 (2015).

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