**Article**

**Epidemiology of Nocardia Species at a Tertiary Hospital in Southern Taiwan, 2012 to 2020: MLSA Phylogeny and Antimicrobial Susceptibility**

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**Abstract**: The identification and antimicrobial susceptibility of *Nocardia* spp. are essential for guiding antibiotic treatment. We investigated the species distribution and evaluated the antimicrobial susceptibility of *Nocardia* species collected in southern Taiwan from 2012 to 2020. A total of 77 *Nocardia* isolates were collected and identified to the species level using multi-locus sequence analysis (MLSA). The susceptibilities to 15 antibiotics for *Nocardia* isolates were determined by the broth microdilution method, and the MIC$_{50}$ and MIC$_{90}$ for each antibiotic against different species were analyzed. *N. cyriacigeorgica* was the leading isolate, accounting for 32.5% of all *Nocardia* isolates, and the prevalence of *Nocardia* isolates decreased in summer. All of the isolates were susceptible to trimethoprim/sulfamethoxazole, amikacin, and linezolid, whereas 90.9% were non-susceptible to cefepime and imipenem. The phylogenetic tree by MLSA showed that the similarity between *N. beijingensis* and *N. asiatica* was as high as 99%, 73% between *N. niigatensis* and *N. crassostreae*, and 86% between *N. cerradoensis* and *N. cyriacigeorgica*. While trimethoprim/sulfamethoxazole, amikacin, and linezolid remained fully active against all of the *Nocardia* isolates tested, 90.9% of the isolates were non-susceptible to cefepime and imipenem.

**Keywords**: nocardiosis; multi-locus sequence analysis; phylogenetic tree analysis; trimethoprim/sulfamethoxazole; imipenem

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**1. Introduction**

Nocardiosis is caused by several species of *Nocardia*, a ubiquitous bacterium in the environment that is transmitted by inhalation or direct cutaneous inoculation [1]. *Nocardia* species are aerobic, partially acid-fast, beaded, branched Gram-positive bacilli with colonies of filamentous, slow-growing, soil-borne bacteria [1,2]. *Nocardia* spp. is responsible for a variety of clinical infections, ranging from skin and soft tissue infections to respiratory and central nervous system infections [3]. Monitoring the epidemiological characteristics of nocardiosis including species distribution, clinical features, and antimicrobial susceptibility profiles is warranted to inform diagnostic and treatment decisions [4].

Different *Nocardia* species may have different geographic distributions, pathogenic characteristics, and antimicrobial susceptibility patterns [5]. Pulmonary nocardiosis usually leads to high mortality and morbidity if not diagnosed in time to initiate the appropriate antimicrobial treatment [6]. Therefore, the identification of *Nocardia* isolates at the species...
level and the determination of their antimicrobial susceptibility are critical for the delivery of appropriate patient care [7].

This study aimed to investigate the species distribution and evaluate the antimicrobial susceptibility patterns of individual *Nocardia* species isolated from patients seeking care at a referral hospital in southern Taiwan from 2012 to 2020. Speciation of *Nocardia* isolates was performed using multi-locus sequence analysis (MLSA).

2. Results

2.1. Patient Characteristics

During the 9-year study period, a total of 77 patients were diagnosed with nocardiosis, 63.6% of whom were male and had an age ranging from 31 to 97 years. The clinical characteristics of the 77 *Nocardia* isolates are shown in Table 1.

Table 1. The clinical characteristics of the 77 included patients.

| Characteristics                  | Gender, n (%) | Age (years) | Specimen type, n (%) | Site of involvement, n (%) |
|----------------------------------|---------------|-------------|----------------------|---------------------------|
| Gender, n (%)                    | Male 49 (63.6)| Median (range) 76 (31–97) | Med 70.4 ± 15.7 | Lung 35 (45.5) |
| Specimen type, n (%)             | Pus 21 (27.3) | Sputum 14 (18.2) | Wound 11 (14.3) | Central nervous system 9 (11.7) |
|                                  | Blood 7 (9.1) | Bronchial washing 7 (9.1) | Corneal ulcer 4 (5.2) | Skin and soft tissue 19 (24.7) |
| Site of involvement, n (%)       | Abscess 5 (6.5) | Pleural effusion 4 (5.2) | Synovial fluid 2 (2.6) | Bone and joint 7 (9.1) |
|                                  | Bone tissue 1 (1.3) | Cerebrospinal fluid 1 (1.3) | Blood stream 7 (9.1) | Disseminated (including blood stream) 18 (23.3) |

2.2. Distribution of Nocardia Species

Of the 77 *Nocardia* isolates, 12 type strains were identified under a phylogenetic tree constructed from the concatenated *gyrB*-16S rRNA*-secA1*-hsp65 sequences. *N. cyriacigeorgica* was the most common species (n = 25, 32.5%), followed by *N. farcinica* (n = 18, 23.4%), *N. brasiliensis* (n = 13, 16.9%), *N. beijingensis* (n = 9, 11.7%), *N. asiatica* (n = 3, 3.9%), *N. asteroides* and *N. concava* (n = 2, 2.6%), *N. amikacinotolerans*, *N. cerradoensis*, *N. crassostreae*, *N. niigatensis*, and *N. otitidiscaviarum* (n = 1, 1.3%).
The correlations between the drug susceptibility patterns and *Nocardia* species are shown in Table 2. The most common drug pattern was type V to type VIII (74.1%). In contrast to the drug pattern types described previously by McTaggart et al. [8], we found that both the *N. farcinica* and *N. cyriacigeorgica* strains were IPM-resistant and the *N. cyriacigeorgica* strains were also FEP-resistant.

Table 2. The antimicrobial susceptibility patterns of different *Nocardia* species.

| Nocardia Species | No. of Isolates | Drug Patterns Types | Antimicrobial Susceptibility Pattern          |
|------------------|----------------|---------------------|---------------------------------------------|
|                  |                | V                   | Non-Susceptible (%) | Susceptible (%) |
| *N. farcinica*   | 18             | V                   | IPM (100)                        | SXT (100)       |
|                  |                |                     | FEP (100)                        | LZD (100)       |
|                  |                |                     | DOX (100)                        | AN (100)        |
|                  |                |                     | TOB (100)                        | CLR (100)       |
| *N. cyriacigeorgica* | 25         | VI                  | CIP (100)                        | SXT (100)       |
|                  |                |                     | IPM (100)                        | LZD (100)       |
|                  |                |                     | MXF (100)                        | AN (100)        |
|                  |                |                     | FEP (100)                        | TOB (100)       |
|                  |                |                     | AMC (100)                        | CLR (92)        |
| *N. brasiliensis* | 13             | VIII                | CIP (100)                        | SXT (100)       |
|                  |                |                     | IPM (100)                        | LZD (100)       |
|                  |                |                     | FEP (100)                        | AN (100)        |
|                  |                |                     | CRO (92)                         | TOB (100)       |
|                  |                |                     | DOX (100)                        | CLR (92)        |
| *N. otitidiscaviarium* | 1          | VII                 | CIP (100)                        | SXT (100)       |
|                  |                |                     | IPM (100)                        | LZD (100)       |
|                  |                |                     | FEP (100)                        | AN (100)        |
|                  |                |                     | AMC (100)                        | TOB (100)       |
|                  |                |                     | CRO (100)                        | CLR (100)       |

Abbreviations: AN, amikacin; AMC, amoxicillin/clavulanic acid 2:1 ratio; CIP, ciprofloxacin; CLR, clarithromycin; CRO, ceftriaxone; DOX, doxycycline; FEP, cefepime; IPM, imipenem; LZD, linezolid; MIN, minocycline; MXF, moxifloxacin; SXT, trimethoprim/sulfamethoxazole; TOB, tobramycin.

2.3. *Nocardia* Species Profile by Analysis of Years and Months

In 2012, 2014, 2019, and 2020, the predominant species was *N. cyriacigeorgica* (Figure 1). In contrast, the predominant species identified in 2013, 2016, 2017, and 2018 was *N. farcinica*. *N. brasiliensis* was the predominant species in 2015. Figure 2 shows the monthly distribution of the *Nocardia* species, suggesting that the prevalence of *Nocardia* infections was lower in summer and higher in autumn.
2.4. Antibiotic Susceptibility Profiles

The MIC$_{50}$ and MIC$_{90}$ values (in µg/mL) and the MIC ranges and distributions for each Nocardia species are shown in Table 3. All Nocardia isolates in our study were susceptible to SXT, AN, and LZD. Of these isolates, 23.4% were non-susceptible to TOB. In contrast, 90.9% of Nocardia isolates were not susceptible to FEP and IPM, especially all isolates of N. cyriacigeorgica, N. farcinica, and N. brasiliensis. We also found that 83.1% of the isolated strains were non-susceptible to CLR, and 80.5% were non-susceptible to CIP. The susceptibility breakpoints for tigecycline and cefoxitin were not established.
Table 3. The antimicrobial susceptibility test results.

| Antimicrobial Agent          | N. cyriacigeorgica (25) | N. brasiliensis (13) | N. farcinica (18) | N. niigatensis (1) | N. asteroides (2) | N. beijerinckia (9) | N. otitidiscaviarum (1) | N. crostostreae (2) | N. concava (2) | N. cerasdoensis (1) | N. asiatica (3) | N. amikacinitorans (1) |
|-----------------------------|------------------------|---------------------|------------------|-------------------|------------------|------------------|------------------|-------------------|----------------|-----------------|----------------|-------------------|
| **Trimethoprim/Sulfamethoxazole (SXT)** |                        |                     |                  |                   |                  |                  |                  |                   |                |                 |                |                   |
| Resistant [n (%)]           | 0                      | 0                   | 0                | 0                 | 0                | 0                | 0                | 0                 | 0              | 0               | 0              | 0                 |
| Intermediate [n (%)]        | 0                      | 0                   | 0                | 0                 | 0                | 0                | 0                | 0                 | 0              | 0               | 0              | 0                 |
| Susceptible [n (%)]         | 25 (100)               | 13 (100)            | 18 (100)         | 1 (100)           | 2 (100)          | 9 (100)          | 1 (100)          | 1 (100)           | 2 (100)         | 1 (100)         | 3 (100)         | 1 (100)           |
| MIC<sub>50</sub> [µg/mL]    | 0.25/4.75              | 0.5/9.5             | 1/19             | 0.25/4.75         | 0.25/4.75        | 0.25/4.75        | 0.25/4.75        | 0.25/4.75         | 0.25/4.75       | 0.25/4.75       | 0.25/4.75       | 0.25/4.75         |
| **Linezolid (LZD)**         |                        |                     |                  |                   |                  |                  |                  |                   |                |                 |                |                   |
| Resistant [n (%)]           | 0                      | 0                   | 0                | 0                 | 0                | 0                | 0                | 0                 | 0              | 0               | 0              | 0                 |
| Intermediate [n (%)]        | 0                      | 0                   | 0                | 0                 | 0                | 0                | 0                | 0                 | 0              | 0               | 0              | 0                 |
| Susceptible [n (%)]         | 25 (100)               | 13 (100)            | 18 (100)         | 1 (100)           | 2 (100)          | 9 (100)          | 1 (100)          | 1 (100)           | 2 (100)         | 1 (100)         | 3 (100)         | 1 (100)           |
| MIC<sub>50</sub> [µg/mL]    | 2                      | 4                   | 4                | 1                 | 2                | 1                | 1                | 1                 | 1              | 1               | 1              | 1                 |
| **Ciprofloxacin (CIP)**     |                        |                     |                  |                   |                  |                  |                  |                   |                |                 |                |                   |
| Resistant [n (%)]           | 25 (100)               | 13 (100)            | 4 (22.2)         | 0                 | 2 (100)          | 5 (55.6)         | 1 (100)          | 1 (100)           | 1 (50)          | 0               | 3 (100)         | 1 (100)           |
| Intermediate [n (%)]        | 0                      | 0                   | 3 (16.7)         | 1 (100)           | 0                | 1 (11.1)         | 0                | 0                 | 1 (50)          | 0               | 0              | 0                 |
| Susceptible [n (%)]         | 0                      | 0                   | 11 (61.1)        | 0                 | 0                | 3 (33.3)         | 0                | 0                 | 0              | 1 (100)         | 0               | 0                 |
| MIC<sub>50</sub> [µg/mL]    | >4                     | >4                  | 1                | 4                 | >4              | >4               | >4               | >4                | >4             | >4              | >4             | >4                |
| **Imipenem (IPM)**          |                        |                     |                  |                   |                  |                  |                  |                   |                |                 |                |                   |
| Resistant [n (%)]           | 21 (84.0)              | 10 (76.9)           | 14 (77.8)        | 1 (100)           | 0                | 5 (55.5)         | 1 (100)          | 1 (100)           | 2 (100)         | 0               | 0              | 1 (100)           |
| Intermediate [n (%)]        | 4 (16.0)               | 3 (23.1)            | 4 (22.2)         | 0                 | 2 (100)          | 1 (11.1)         | 0                | 0                 | 0              | 0               | 0              | 0                 |
| Susceptible [n (%)]         | 0                      | 0                   | 0                | 0                 | 0                | 3 (33.3)         | 0                | 0                 | 0              | 1 (100)         | 3 (100)         | 0                 |
| MIC<sub>50</sub> [µg/mL]    | 16                     | 32                  | 16               | 16                | 16              | 16               | 16               | 16                | 16             | 16              | 16             | 16                |
| **Moxifloxacin (MXF)**      |                        |                     |                  |                   |                  |                  |                  |                   |                |                 |                |                   |
| Resistant [n (%)]           | 24 (96.0)              | 10 (76.9)           | 14 (77.8)        | 1 (100)           | 0                | 5 (55.5)         | 1 (100)          | 1 (100)           | 2 (100)         | 0               | 0              | 1 (100)           |
| Intermediate [n (%)]        | 1 (4.0)                | 1 (7.8)             | 0                | 1 (50)            | 2 (22.3)         | 0                | 0                | 0                 | 0              | 1 (100)         | 0               | 0                 |
| Susceptible [n (%)]         | 0                      | 2 (15.4)            | 14 (77.8)        | 1 (100)           | 0                | 4 (44.4)         | 1 (100)          | 2 (100)           | 0              | 3 (100)         | 0               | 0                 |
| MIC<sub>50</sub> [µg/mL]    | 4                      | 0.25                | 0.25             | 0.25              | 0.25            | 0                | 0                | 0                 | 0              | 8               | 8              | 8                 |
| **Cefepime (FEP)**          |                        |                     |                  |                   |                  |                  |                  |                   |                |                 |                |                   |
| Resistant [n (%)]           | 16 (64.0)              | 12 (92.3)           | 16 (88.8)        | 1 (100)           | 2 (100)          | 4 (44.4)         | 1 (100)          | 1 (100)           | 2 (100)         | 0               | 0              | 1 (100)           |
| Intermediate [n (%)]        | 9 (36.0)               | 1 (7.8)             | 2 (11.2)         | 0                 | 0                | 2 (22.3)         | 0                | 0                 | 0              | 0               | 0              | 0                 |
| Susceptible [n (%)]         | 0                      | 0                   | 0                | 0                 | 0                | 3 (33.3)         | 0                | 0                 | 0              | 1 (100)         | 3 (100)         | 0                 |
| MIC<sub>50</sub> [µg/mL]    | 32                     | >32                 | >32              | >32               | >32            | >32              | >32             | >32               | >32           | 16              | 8              | 8                 |
| **Cefoxitin (FOX)**         |                        |                     |                  |                   |                  |                  |                  |                   |                |                 |                |                   |
| MIC range                   | 64–128                 | 16–128              | 64–128           | >128              | 16–64           | 8–32             | >128            | >128              | >128          | 64              | 4–16           | >64              |
Table 3. Cont.

| Antimicrobial Agent       | Species (No. of Strains Tested) | N. cyriacigeorgica (25) | N. brasiliensis (13) | N. farcinica (18) | N. niigatensis (1) | N. asteroides (2) | N. beijingensis (9) | N. ositisdiscaevianum (1) | N. conca (2) | N. cerasostreae (1) | N. concava (2) | N. cassidisovari (1) | N. beijingensis (9) | N. otitidisovari (1) |
|---------------------------|---------------------------------|-------------------------|----------------------|-------------------|-------------------|-------------------|-------------------|------------------------|----------------|-------------------|----------------|---------------------|-------------------|---------------------|
| Amoxicillin/clavulanic acid 2:1 ratio (AMC) | Resistant [n (%)] | 23 (92.0) | 2 (15.4) | 12 (66.6) | 0 | 0 | 2 (100) | 4 (44.5) | 1 (100) | 1 (100) | 2 (100) | 0 | 3 (100) | 0 |
| Intermediate [n (%)] | 2 (8.0) | 1 (7.7) | 12 (66.6) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Susceptible [n (%)] | 0 | 0 | 3 (16.7) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MICs [µg/mL] | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 |
| Amikacin (AN) | Resistant [n (%)] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Intermediate [n (%)] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Susceptible [n (%)] | 25 (100) | 13 (100) | 18 (100) | 1 (100) | 2 (100) | 9 (100) | 1 (100) | 1 (100) | 2 (100) | 1 (100) | 3 (100) | 1 (100) | 0 | 0 |
| MICs [µg/mL] | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 |
| Ceftriaxone (CRO) | Resistant [n (%)] | 2 (8.0) | 10 (76.9) | 15 (83.3) | 1 (100) | 0 | 1 (100) | 1 (100) | 1 (100) | 2 (100) | 0 | 0 | 1 (100) | 0 |
| Intermediate [n (%)] | 8 (32.0) | 2 (15.4) | 1 (5.6) | 0 | 0 | 3 (33.3) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Susceptible [n (%)] | 25 (100) | 13 (100) | 18 (100) | 1 (100) | 2 (100) | 9 (100) | 1 (100) | 1 (100) | 2 (100) | 1 (100) | 3 (100) | 1 (100) | 0 | 0 |
| MICs [µg/mL] | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 |
| Doxycycline (DOX) | Resistant [n (%)] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Intermediate [n (%)] | 17 (68.0) | 12 (92.3) | 17 (94.4) | 1 (100) | 2 (100) | 6 (66.7) | 1 (100) | 1 (100) | 0 | 1 (100) | 0 | 1 (100) | 1 (100) |
| Susceptible [n (%)] | 8 (32.0) | 4 | 4 | 4 | 3 (33.3) | 0 | 0 | 0 | 0 | 3 (100) | 1 (100) | 0 | 0 |
| MICs [µg/mL] | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 |
| Minocycline (MIN) | Resistant [n (%)] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Intermediate [n (%)] | 17 (68.0) | 11 (84.6) | 17 (94.4) | 1 (100) | 2 (100) | 3 (33.3) | 1 (100) | 1 (100) | 0 | 1 (100) | 0 | 1 (100) | 1 (100) |
| Susceptible [n (%)] | 8 (32.0) | 2 (15.4) | 1 (5.6) | 0 | 0 | 6 (66.7) | 0 | 0 | 0 | 0 | 3 (100) | 1 (100) | 0 | 0 |
| MICs [µg/mL] | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 | >64/32 |
| Tigecycline (TGC) | MIC range | 0.25-2 | 0.25-0.5 | 0.5-4 | 0.5-1 | 0.12-0.5 | 0.5-1 | 2 | 2 | 2 (100) | 0.12 | 0.25 | 2 |
| Tobramycin (TOB) | MICs | 0.25-2 | 0.25-0.5 | 0.5-4 | 0.5-1 | 0.12-0.5 | 0.5-1 | 2 | 2 | 2 (100) | 0.12 | 0.25 | 2 |
| Clarithromycin (CLR) | Resistant [n (%)] | 22 (88.0) | 9 (69.2) | 18 (100) | 1 (100) | 2 (100) | 2 (22.2) | 1 (100) | 1 (100) | 0 | 0 | 1 (33.3) | 1 (100) |
| Intermediate [n (%)] | 2 (8.0) | 1 (7.8) | 0 | 0 | 5 (55.6) | 0 | 0 | 0 | 2 (100) | 1 (100) | 2 (66.7) | 0 |
| Susceptible [n (%)] | 1 (4.0) | 3 (23.0) | 0 | 0 | 2 (22.2) | 0 | 0 | 0 | 0 | 1 (100) | 0 | 0 |
| MICs [µg/mL] | >16 | >16 | >16 | >16 | >16 | >16 | >16 | >16 | >16 | >16 | >16 | >16 | >16 |

A comparison of the activities of different antibiotics against N. cyriacigeorgica and N. farcinica revealed that the MICs of MXF and AMC against N. cyriacigeorgica were higher than those against N. farcinica. In contrast, the MICs of SXT, LZD, IPM, CRO, and TGC against N. cyriacigeorgica were lower than those against N. farcinica (Table 3).
2.5. PFGE for N. cyriacigeorgica

N. cyriacigeorgica was the most common species in this study. We randomly selected nine strains of N. cyriacigeorgica, which were isolated in 2019 and 2020 for PFGE analysis to determine the genetic relatedness among the strains. Figure 3 shows that all nine strains were isolated from different patients, and their parental similarities were less than 60%, indicating non-homologous strains.

![Figure 3. Genetic relationships of N. cyriacigeorgica by PFGE analysis.](image)

2.6. Phylogenetic Tree by MLSA Scheme

In our study, there were 12 Nocardia spp. The differences in four-locus (gyrB-16S rRNA-secA1-hsp65) MLSA concatenated sequences among these 12 species and the evolutionary phylogenetic trees are shown in Figure 4. The similarity between N. beijingensis and N. asiatica was as high as 99%, and that between the two species and N. farcinica was 77%. N. beijingensis and N. asiatica belonged to the N. abscessus complex [7], and their nucleic acid similarity was up to 99%. N. niigatensis and N. crassostreae had a similarity of 73%. N. cerradoensis had an 86% similarity to N. cyriacigeorgica. Additionally, N. amikacinitolerae was independent and had greater differences than the other species.

![Figure 4. A phylogenetic neighbor-joining tree including these 12 types of strains as an indicator and the 77 clinical strains studied in the nine years based on the MLSA concatenated sequence.](image)
3. Discussion

Recently, MALDI-TOF MS has been shown to provide an accurate identification of Nocardia species when an augmented Nocardia library is employed. However, while some species are easily identified (i.e., N. brasiliensis), for others, the identification has only been shown to extend to the complex level (N. abscessus complex, N. brevicatena-N. paucivorans complex, N. nova complex, and N. transvalensis complex). The identification of uncommon species remains a challenge [7,9]. Sequence analysis of the 16S rRNA gene is suggested as the “gold standard” for the identification of Nocardia isolates to the species level. However, when the identification to species level is based on the partial 5’ 16S rRNA sequencing, as in this case, a second genetic locus such as the secA gene for isolate identification is recommended because 16S rRNA sequence analysis alone provides insufficient species-level resolution for many Nocardia spp., whereas secA gene sequence analysis is more discriminatory and gives better resolution to the species level [10]. Both genes were included in the MLSA schema employed in this study for the species assignation to achieve higher accuracy and differentiation. Nevertheless, the identification of Nocardia isolates in some challenging species, species groups, or complexes is not possible with MLSA. In our study, there was a 99% similarity between N. beijingensis and N. asiatica in the MLSA analysis, and all of them belonged to the N. abscessus complex, which was difficult to distinguish.

Previous studies conducted before 2010 indicated that the most common Nocardia spp. in Taiwan was N. brasiliensis [11,12]. In contrast, N. farcinica was the most common isolated species in China from 2009 to 2021 [13]. N. nova complex organisms were the most common isolates in the United States before 2004 and Canada before 2008 [14,15], and N. cyriacigeorgica was the most common pathogen in Spain before 2008 [16]. N. cyriacigeorgica was the most common causative agent of pulmonary nocardiosis in southern Taiwan from 2004 to 2010 [17] and China from 2010 to 2020, where pulmonary nocardiosis (90.2%) was the most common clinical presentation of infection [18], which is consistent with our study predominated in lung infection (Table 1) conducted between 2012 and 2020. There are few recent epidemiological data on invasive nocardiosis in this region. Further studies are required to confirm whether N. cyriacigeorgica is an emerging pathogen in southern Taiwan.

The different species of Nocardia isolates exhibit diverse susceptibilities to antibiotics. Our study showed that all Nocardia spp. are susceptible to SXT, LZD, and AN. In contrast, the non-susceptibility rates of Nocardia spp. to DOX and MIN were 80.5% and 71.4%, respectively. Overall, SXT, LZD, and AN were the most active drugs for all Nocardia spp., which is consistent with the findings of other studies [8,10,19]. Our study showed that all Nocardia spp. were susceptible to TOB, except for N. farcinica. This suggests that TOB should be avoided in infections with N. farcinica in our region.

Although our study showed that most of the drug patterns were consistent with the drug pattern types suggested by McTaggart [8], different antibiograms were found in the current study. In agreement with the report of Tan et al. [10], high IPM resistance rates were observed in both N. farcinica and N. cyriacigeorgica, and high FEP resistance rates were observed in N. cyriacigeorgica in our study (Table 3). High IPM resistance in N. cyriacigeorgica was observed in Australia [20], but not in Spain [19] and Canada [8]. Another study of 151 Nocardia isolates conducted in four hospitals in Taiwan between 1998 and 2009 found that the three leading Nocardia spp. were N. brasiliensis, N. cyriacigeorgica, and N. farcinica. The susceptibility of N. brasiliensis, N. cyriacigeorgica, and N. farcinica to IPM was 47%, 100%, and 100%, respectively [11]. The higher rate of non-susceptibility of IPM observed in our study could either be a unique regional resistance profile of Nocardia spp. in southern Taiwan or selection pressure from the overuse of carbapenems [21,22]. This finding suggests that FEP and IPM should not be used empirically until the antimicrobial susceptibility results are available. Further epidemiological surveillance of the antimicrobial susceptibility profiles of Nocardia spp. is warranted to confirm our findings.

Nocardiosis occurs worldwide. Nocardia infections have increased in the past decades, likely due to improved detection and identification methods and the expanding im-
munocompromised population [3]. Although reports of community-acquired nocardiosis are common, few cases of nosocomial transmission of Nocardia species have been reported [23–26]. N. cyriacigeorgica has also been reported to cause outbreaks [27]. We performed a PFGE analysis for N. cyriacigeorgica, and the genetic relatedness of the strains from different patients were not homologous (Figure 3). Remarkably, no outbreaks occurred in this study. Our finding of a decrease in the prevalence of clinically isolated Nocardia spp. in summer from 2012 to 2020 is in contrast to the findings of an Australian environmental survey of Nocardia species isolated during a 1-year period from the foaming marine waters of the Sunshine Coast region [28], which suggests that hot weather is conducive to the growth of Nocardia. However, more studies of the prevalence of Nocardia species among clinical samples per month are needed to gain insights into the correlation of climate change and the distribution of Nocardia spp.

Our study had some limitations. First, the number of isolated Nocardia spp. in this study was still low, which may have prevented us from exactly determining the prevalence. Second, we did not investigate the molecular mechanisms of the antimicrobial resistance of the collected Nocardia strains to explain the regional differences in the antimicrobial susceptibility profiles.

4. Materials and Methods
4.1. Bacterial Isolates

Non-duplicated 77 isolates of Nocardia spp. collected from all patients who received a culture-confirmed diagnosis of nocardiosis at Kaohsiung Chang Gung Memorial Hospital (KCGMH) were included from 1 January 2012 to 31 December 2020. The KCGMH is a 2700-bed facility that serves as a primary care and tertiary referral center in southern Taiwan.

4.2. Housekeeping Gene Selection, DNA Extraction, PCR, and Sequencing

According to previous studies, four housekeeping genes (16S rRNA, secA1, gyrB, and hsp65) were selected [29,30]. DNA was extracted using a QIAGEN DNeasy Tissue Kit. The PCR products were referred to the Genome Sequencing Company for sequencing. The gene sequences were subsequently matched to those in the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov, accessed on 21 November 2021) to identify the Nocardia species [14,29]. The gene sequences were deposited in the GenBank database and their corresponding accession numbers are presented in Table S1.

4.3. Construction of Phylogenetic Tree

MLSA using concatenated sequences of gyrB-16S-secA1-hsp65 has previously been used to identify Nocardia species [29,30]. Primer sequences published by McTaggart et al. [31] are presented in Table S2. Phylogenetic trees were constructed using the neighbor-joining method (software: Molecular Evolutionary Genetics Analysis across Computing Platforms). Bootstrap values based on 1000 replications were listed as percentages at the branching points of the tree [32]. Phylogenetic trees were constructed using the neighbor-joining (NJ) genetic distance method [33] and performed using the ClustalW algorithm in the mega X software. The reliability of each tree topology was checked using 10,000 bootstrap replications [32,34].

4.4. Pulsed-Field Gel Electrophoresis (PFGE) Analysis

N. cyriacigeorgica was the most common species in this study, so we performed PFGE to clarify whether there was a possibility of nosocomial infection. The suspension (300 µL) and lysozyme (20 µL; 25 mg/mL) were added and incubated at 37 °C for 4 h after mixing. Total genomic DNA was prepared in agarose plugs and lysed in 5 mL of lysis buffer (25 mg lysozyme per mL and 20 µL proteinase K in TE buffer) for 4 h in a 56 °C water bath. The plugs were digested with XbaI. DNA fragments were separated on a 1% gel in a CHEF Mapper System (Bio-Rad, Mississauga, Ontario, Canada) with linear ramping pulse times
of 1–30 s over 17.5, 6 V/cm at 14 °C. The Dice coefficients of the PFGE profiles were analyzed with an UPGMA dendrogram using GelCompar II version 6.6.11 (Applied Maths BVBA, Kortrijk, Belgium).

4.5. Antimicrobial Susceptibility Test

The susceptibility of the isolates to 15 commonly-used antibiotics was tested by the microbroth dilution method using Sensititre RAPMYCO TREK (Sensititre Susceptibility plates; TREK Diagnostic Systems Ltd. Cleveland, OH, USA) according to the manufacturer’s instructions. The strains recommended by the CLSI, *S. aureus* ATCC 29213 and *E. coli* ATCC 25922, were tested for quality control.

Antibiotics chosen for susceptibility testing in this study included amikacin (AN), amoxicillin/clavulanic acid (AMC), cefepime (FEP), cefoxitin (FOX), ceftriaxone (CRO), ciprofloxacin (CIP), clarithromycin (CLR), doxycycline (DOX), imipenem (IPM), linezolid (LZD), minocycline (MIN), moxifloxacin (MXF), tigecycline (TGC), tobramycin (TOB), and trimethoprim/sulfamethoxazole (SXT). The results were interpreted according to CLSI guideline M62 for aerobic actinomycetes [35].

4.6. Antimicrobial Susceptibility Patterns

According to Wallace et al. [36], six patterns of antibiotic susceptibility to *Nocardia* spp. have been proposed. These include *N. abscessus* complex (drug pattern I) and *N. brevicatena/N. paucivorans* (drug pattern II), *Nocardia nova* complex (drug pattern III), *Nocardia transvalensis* complex (drug pattern IV), *N. farcinica* (drug pattern V), and *N. cyriacigeorgica* (drug pattern VI) [36,37]. McTaggart et al. [8] suggested numerous rarely-occurring species using broth microdilution and divided them into four other drug patterns. We also characterized the antimicrobial resistance of several *Nocardia* isolates and profiled their antimicrobial susceptibility patterns.

5. Conclusions

*N. cyriacigeorgica* was the major *Nocardia* spp. identified in this study. SXT, LZD, and AN were the most active antimicrobial agents against all *Nocardia* strains identified. The distribution and antibiotic resistance characteristics of *Nocardia* species further our understanding of the diversity of circulating *Nocardia* species and inform the decision-making in the choice of empirical therapy.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/antibiotics11101438/s1, Table S1: Accession number for four genes of these 12 type strains of Nocardia species as an indicator from GenBank, Table S2: Housekeeping genes primer.

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Informed Consent Statement: Patient consent was waived for this study because only anonymous data were retrospectively analyzed and published.

Data Availability Statement: The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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References

1. Brown-Elliott, B.A.; Brown, J.M.; Convillo, P.S.; Wallace, R.J., Jr. Clinical and laboratory features of the Nocardia spp. based on current molecular taxonomy. Clin. Microbiol. Rev. 2006, 19, 259–282. [CrossRef] [PubMed]
2. Duggal, S.D.; Chugh, T.D. Nocardiosis: A neglected disease. Med. Prin. Pract. 2020, 29, 514–523. [CrossRef] [PubMed]
3. Williams, E.; Jenney, A.W.; Spelman, D.W. Nocardia bacteremia: A single-center retrospective review and a systematic review of the literature. Int. J. Infect. Dis. 2020, 92, 197–207. [CrossRef] [PubMed]
4. Huang, L.; Chen, X.; Xu, H.; Sun, L.; Li, C.; Guo, W.; Xiang, L.; Luo, G.; Cui, Y.; Lu, B. Clinical features, identification, antimicrobial resistance patterns of Nocardia species in China: 2009–2017. Diagn. Microbiol. Infect. Dis. 2019, 94, 165–172. [CrossRef]
5. Lebeaux, D.; Bergeron, E.; Berthet, J.; Djadi-Prat, J.; Mounié, D.; Boiron, P.; Lortholary, O.; Rodriguez-Nava, V. Antibiotic susceptibility testing and species identification of Nocardia isolates: A retrospective analysis of data from a French expert laboratory, 2010–2015. Clin. Microbiol. Infect. 2019, 25, 489–495. [CrossRef]
6. Van den Bogaart, L.; Manuel, O. Antibiotic therapy for difficult-to-treat infections in lung transplant recipients: A practical approach. Antibiotics 2022, 11, 612. [CrossRef]
7. Convillo, P.S.; Brown-Elliott, B.A.; Smith, T.; Zelazny, A.M. The complexities of Nocardia taxonomy and identification. J. Clin. Microbiol. 2017, 56, e01419-17. [CrossRef]
8. McTaggart, L.R.; Doucet, J.; Witkowska, M.; Richardson, S.E. Antimicrobial susceptibility among clinical Nocardia species identified by multilocus sequence analysis. Antimicrob. Agents Chemother. 2015, 59, 269–275. [CrossRef]
9. Marin, M.; Ruiz, A.; Iglesias, C.; Quiroga, L.; Cercenado, E.; Martin-Rabadán, P.; Bouza, E.; Rodriguez-Sánchez, B. Identification of Nocardia species from clinical isolates using MALDI-TOF mass spectrometry. Clin. Microbiol. Infect. 2018, 24, 1342.e5–1342.e8. [CrossRef]
10. Tan, Y.E.; Chen, S.C.; Halliday, C.L. Antimicrobial susceptibility profiles and species distribution of medically relevant Nocardia species: Results from a large tertiary laboratory in Australia. J. Glob. Antimicrob. Resist. 2020, 20, 110–117. [CrossRef]
11. Lai, C.C.; Liu, W.L.; Ko, W.C.; Chen, Y.H.; Tan, H.R.; Huang, Y.T.; Hsueh, P.R. Multicenter study in Taiwan of the in vitro activities of nemonoxacin, tigecycline, doripenem, and other antimicrobial agents against clinical isolates of various Nocardia species. Antimicrob. Agents Chemother. 2011, 55, 2084–2091. [CrossRef]
12. Liu, W.L.; Lai, C.C.; Ko, W.C.; Chen, Y.H.; Tang, H.J.; Huang, Y.L.; Huang, Y.T.; Hsueh, P.R. Clinical and microbiological characteristics of infections caused by various Nocardia species in Taiwan: A multicenter study from 1998 to 2010. Eur. J. Clin. Microbiol. Infect. Dis. 2011, 30, 1341–1347. [CrossRef]
13. Wang, H.; Zhu, Y.; Cui, Q.; Wu, W.; Li, G.; Chen, D.; Xiang, L.; Qu, J.; Shi, D.; Lu, B. Epidemiology and Antimicrobial Resistance Profiles of the Nocardia species from 2009 to 2021. Microbiol. Spectr. 2022, 10, e0156021. [CrossRef]
14. Tremblay, J.; Thibert, L.; Alarie, I.; Valiquette, L.; Pépin, J. Nocardiosis in Quebec, Canada, 1988–2008. Clin. Microbiol. Infect. 2011, 17, 690–696. [CrossRef]
15. Uhde, K.B.; Pathak, S.; McCullum, I.; Janmat-Khah, D.P.; Jr.; Shadomy, S.V.; Dykewicz, C.A.; Clark, T.A.; Smith, T.L.; Brown, J.M. Antimicrobial-resistant Nocardia isolates, United States, 1995–2004. Clin. Infect. Dis. 2010, 51, 1445–1448. [CrossRef]
16. Minero, M.V.; Marin, M.; Cercenado, E.; Rabadán, P.M.; Bouza, E.; Muñoz, P. Nocardiosis at the turn of the century. Medicine 2009, 88, 250–261. [CrossRef]
17. Chen, Y.C.; Lee, C.H.; Chien, C.C.; Chao, T.L.; Lin, W.C.; Liu, J.W. Pulmonary nocardiosis in southern Taiwan. J. Microbiol. Immunol. Infect. 2013, 46, 441–447. [CrossRef]
18. Wei, M.; Xu, X.; Yang, J.; Wang, P.; Liu, Y.; Yang, S.; Yang, C.; Gu, L. MLSA phylogeny and antimicrobial susceptibility of clinical Nocardia isolates: A multicenter retrospective study in China. BMC Microbiol. 2021, 21, 342. [CrossRef]
19. Valdezate, S.; Garrido, N.; Carrasco, G.; Medina-Pascual, M.J.; Villalon, P.; Navarro, A.M.; Saéz-Nieto, J.A. Epidemiology and susceptibility to antimicrobial agents of the main Nocardia species in Spain. J. Antimicrob. Chemother. 2017, 72, 754–761. [CrossRef]
20. McGuinness, S.L.; Whiting, S.E.; Baird, R.; Currie, B.J.; Ralph, A.P.; Anstey, N.M.; Price, R.N.; Davis, J.S.; Tong, S.Y.C. Nocardiosis in the tropical northern territory of Australia, 1997–2014. Open Forum Infect. Dis. 2016, 3, ofw208. [CrossRef]
21. Grau, S.; Fondevilla, E.; Echeverria-Esnal, D.; Alcorta, A.; Limon, E.; Gudiol, F.; VINCat Program group. Widespread increase of carbapenem use in acute care hospitals in Catalonia, Spain. Enferm. Infect. Microbiol. Clin. 2019, 37, 36–40. [CrossRef]
22. Rhodes, N.J.; Wagner, J.L.; Davis, S.L.; Bosso, J.A.; Goff, D.A.; Rybak, M.J.; Scheetz, M.H.; MAD-ID Research Network. Trends in and predictors of carbapenem consumption across north American hospitals: Results from a multicenter survey by the MAD-ID research network. Antimicrob. Agents Chemother. 2019, 63, e00327-19. [CrossRef]
23. Yew, W.W.; Wong, P.C.; Kwan, S.Y.; Chan, C.Y.; Li, M.S. Two cases of Nocardia asteroides sternotomy infection treated with ofloxacin and a review of other active antimicrobial agents. J. Infect. 1991, 23, 297–302. [CrossRef]
24. Exmelin, L.; Malbruny, B.; Vergnaud, M.; Prosvost, F.; Boiron, P.; Morel, C. Molecular study of nosocomial nocardiosis outbreak involving heart transplant recipients. *J. Clin. Microbiol.* **1996**, *34*, 1014–1016. [CrossRef]

25. Blümel, J.; Blümel, E.; Yassin, A.F.; Schmidt-Rotte, H.; Schaal, K.P. Typing of *Nocardia farcinica* by pulsed-field gel electrophoresis reveals an endemic strain as source of hospital infections. *J. Clin. Microbiol.* **1998**, *36*, 118–122. [CrossRef]

26. Wenger, P.N.; Brown, J.M.; McNeil, M.M.; Jarvis, W.R. *Nocardia farcinica* sternotomy site infections in patients following open heart surgery. *J. Infect. Dis.* **1998**, *178*, 1539–1543. [CrossRef]

27. Apostolou, A.; Bolcen, S.J.; Dave, V.; Jani, N.; Lasker, B.A.; Tan, C.G.; Montana, B.; Brown, J.M.; Genese, C.A. *Nocardia cyriacigeorgica* infections attributable to unlicensed cosmetic procedures—An emerging public health problem? *Clin. Infect. Dis.* **2012**, *55*, 251–253. [CrossRef]

28. Wright, L.; Katouli, M.; Kurtböke, D.I. Isolation and characterization of *Nocardiae* associated with foaming coastal marine waters. *Pathogens* **2021**, *10*, 579. [CrossRef] [PubMed]

29. Takeda, K.; Kang, Y.; Yazawa, K.; Gomi, T.; Mikami, Y. Phylogenetic studies of *Nocardia* species based on gyrB gene analyses. *J. Med. Microbiol.* **2010**, *59*, 165–171. [CrossRef] [PubMed]

30. Kong, F.; Wang, H.; Zhang, E.; Sintchenko, V.; Xiao, M.; Sorrell, T.C.; Chen, X.; Chen, S.C. secA1 gene sequence polymorphisms for species identification of *Nocardia* species and recognition of intraspecies genetic diversity. *J. Clin. Microbiol.* **2010**, *48*, 3928–3934. [CrossRef] [PubMed]

31. McTaggart, L.R.; Richardson, S.E.; Witkowska, M.; Zhang, S.X. Phylogeny and identification of *Nocardia* species on the basis of multilocus sequence analysis. *J. Clin. Microbiol.* **2010**, *48*, 4525–4533. [CrossRef]

32. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549. [CrossRef]

33. Gascuel, O. BIONJ: An improved version of the NJ algorithm based on a simple model of sequence data. *Mol. Biol. Evol.* **1997**, *14*, 685–695. [CrossRef]

34. Gnanam, H.; Rajapandian, S.; Gunasekaran, R.; Roshni Prithiviraj, S.; Ravindran, R.S.; Sen, S.; Prajna, L. Molecular identification of *Nocardia* species causing endophthalmitis using multilocus sequence analysis (MLSA): A 10-year perspective. *J. Med. Microbiol.* **2020**, *7*, 728–738. [CrossRef]

35. Clinical and Laboratory Standards Institute. *Performance Standards for Susceptibility Testing of Mycobacteria, Nocardia spp., and Other Aerobic Actinomycetes*, 1st ed.; Approved standard M62; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2018.

36. Wallace, R.J.; Steele, L.C., Jr.; Sumter, G.; Smith, J.M. Antimicrobial susceptibility patterns of *Nocardia asteroides*. *Antimicrob. Agents Chemother.* **1988**, *32*, 1776–1779. [CrossRef]

37. Zhao, P.; Zhang, X.; Du, P.; Li, G.; Li, L.; Li, Z. Susceptibility profiles of *Nocardia* spp. to antimicrobial and antituberculous agents detected by a microplate Alamar Blue assay. *Sci. Rep.* **2017**, *7*, 43660. [CrossRef]