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Clinical features, identification, antimicrobial resistance patterns of *Nocardia* species in China: 2009–2017

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ABSTRACT

Nocardia spp. is a pathogen responsible for a variety of clinical infections, ranging from skin and soft tissue infections, to the respiratory tract and central nervous system infections. Its epidemiological characteristics, including species distribution, clinical features, and antimicrobial susceptibility profiles, should be under surveillance for the prevention and treatment of nocardiosis. In the present study, over a 9-year period (from 2009 to 2017), 53 non-repetitive *Nocardia* isolates were collected from 8 tertiary general hospitals of 7 cities in China. These isolates were identified to species level by multilocus sequence analysis(MLSA). The clinical data were also reviewed. The susceptibilities to 10 commonly-used antibiotics for *Nocardia* were determined by E-test stripes, and the resistance rates, MIC_{50} and MIC_{90} to each antibiotic by different species were analyzed. Of 53 *Nocardia* isolates, *N. farcinica* was the most common species (24.5%, 13/53), followed by *N. cyriacigeorgica* (20.8%, 11/53), *N. terpenica* (15.1%, 8/53), *N. abscessus* (9.43%, 5/53), *N. otitidiscaviarum* (7.55%, 4/53), respectively. Furthermore, 31 *Nocardia* (58.5%) isolates were recovered from lower respiratory tract (sputum and BALF), 15 (28.3%) from superficial Infection, 3 (5.7%) from pleural effusion, 2 (3.8%) from CSF, and 1 from bone marrow and 1 from synovial fluid, respectively. The antibiotic resistance profiles varied between different *Nocardia* species. All *Nocardia* isolates were susceptible to linezolid, followed by imipenem and amikacin (both 92.5% susceptibility rate). *N. terpenica*, rarely documented elsewhere, showed a different antimicrobial susceptibility profile. In summary, herein, the clinical and antibiotic resistance features of *Nocardia* species reported would be helpful for understanding the diversity of *Nocardia* species circulating in China and for decision making in the context of empiric therapy.

1. Introduction

The genus *Nocardia* are ubiquitous gram-positive, aerobic and slow-growing actinomycetes in the environment and can cause opportunistic infections (Ambrosioni et al., 2010; Lebeaux et al., 2018), ranging from localized cutaneous and pulmonary infections, to deep-seated or disseminated infections in immunosuppressed patients (Brown-Elliott et al., 2006; Lebeaux et al., 2018; Rafiei et al., 2016). Nocardiosis has been increasingly reported in recent years worldwide (Valdezate et al., 2017; Yi et al., 2014).

The distribution and the data of susceptibility profiles available worldwide showed geographic variation and changed over time (Lai et al., 2011; Uhde et al., 2010). The susceptibility patterns of *Nocardia*, defined previously (Brown-Elliott et al., 2006; McTaggart et al., 2015), are also helpful for classification within a *Nocardia* species or group, which is often a tough job in routine microbiology laboratories (McTaggart et al., 2010). However, the characteristics of nocardiosis in mainland China are scantly described. Only a few reports can be found in recent years (Wei et al., 2017; Yang et al., 2014), often with a small sample size or limited resources of *Nocardia* isolates. Therefore, we included 53 clinical isolates of *Nocardia* species recovered from 7 cities in China in the current study. To the best of our knowledge, this is the first comprehensive evaluation of the clinical features, species

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identification, distribution, and antibiotic susceptibility profiles of *Nocardia* in mainland China.

2. Materials and methods

2.1. Strains collection and identification

During the period from 2009 to 2017, 53 non-repetitive isolates of *Nocardia* were collected from clinical specimens from 8 hospitals in 7 cities in China. Among them, 18 were from Peking University First Hospital, Beijing (2009–2017), 5 from Civil Aviation General Hospital, Beijing (2013–2017), 10 from Xiamen University First Affiliated Hospital, Xiamen, Fujian province (2016–2017), 12 from People's hospital of Guangxi Zhuang Autonomous Region, Nanning, Guangxi province (2016–2017), 3 from Liuyang Hospital of Chinese Medicine, Liuyang, Hunan province (2017), 2 from Affiliated Hospital of Chongqing medical and Pharmaceutical College, Chongqing (2015–2017), 2 from Weifang People's hospital, Weifang, Shandong province (2016), and 1 from Fourth-affiliated hospital of Guangxi Medical University, Liuzhou, Guangxi Zhuang Autonomous Region (2017).

The bacteria isolation and culture protocol were performed as described previously (Larruskain et al., 2011). Briefly, the clinical specimens, including bronchoalveolar lavage fluid (BALF), expectorated sputum, pleural effusion, cerebrospinal fluid (CSF), skin and soft tissue pus, *etc*., were inoculated onto Columbia blood agar plates, and cultured at 35 °C incubator for 3–7 days. The morphology of bacteria colony from each plate was inspected every day. The presumptive identification was based on both colony morphology and modified acid-fast stain. All these strains were forwarded to Department of Clinical Laboratory, Peking University First Hospital. Definitive identification was performed by sequencing full length of 16S rRNA gene, then complemented with sequencing of the heat shock protein 65 (*hsp*65) gene, the subunit A of SecA preprotein translocase (secA1) gene, RNA polymerase (*rpo*B), and DNA gyrase subunit B (*gyr*B) gene. These 5 sets of primer sequences and PCR reaction conditions were described previously (Baio et al., 2013; Conville et al., 2006; Takeda et al., 2010; Yin et al., 2007). The PCR products were purified and sequencing was performed by using ABI 3730 DNA analyzer. The sequences were compared to the database in NCBI Genbank (http://www.ncbi.nlm.nih.gov) using BLAST algorithm. Species identification was based on the similarity value of ≥99.6% for 16S rRNA, ≥99.0% for secA1, ≥93.5% for *gyr*B (Takeda et al., 2010; Tremblay et al., 2011). If there are discrepant results in identification, the *Nocardia* species will be determined using multilocus sequence analysis (MLSA), as previously described (McTaggart et al., 2010).

2.2. Definition of invasive and non-invasive infection

Invasive infection was defined as isolation of *Nocardia* strains from normally-sterile site (*e.g.* CSF, pleural effusion), while noninvasive infection was defined as isolation of *Nocardia* from normally nonsterile sites with a clinical syndrome consistent with *Nocardia* infection in accordance with our previous study (Lu et al., 2017).

2.3. Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed by using E-test (BioMerieux, Marcy-l'Etoile, France), as described previously for *Nocardia* spp. (Ambaye et al., 1997; Glupczynski et al., 2006). The choice of various antibiotics was based on the first-line and second-line drugs used for *Nocardia* provided in CLSI M24-2A, including amikacin, trimethoprim-sulfamethoxazole (TMP-SMX), gentamicin, ciprofloxacin, cefepime, imipenem, ceftriaxone, amoxicillin-clavulanate, linezolid, and cefotaxime. Furthermore, the antibiotic susceptibility testing was performed as described previously (Ambaye et al., 1997; Glupczynski et al., 2006; Institute, 2011). Briefly, the colonies of those identified isolates growing on the blood agar were collected and transferred into sterile 0.9% sodium chloride water, then subjected to repeated vortexing. The supernatant was adjusted to the turbidity of 1.0 McFarland. The samples were then spread onto Muller-Hinton blood agar plates, and the MICs were determined following the instructions of E-test stripe after 48–72 hours of incubation at 35 °C. The MIC was defined as the value at which inhibition zone intercepted the scale on E-test stripe, as documented elsewhere (Valdezate et al., 2017). The antibiotic susceptibility were judged as susceptible, intermediate or resistant based on CLSI M24-2A (Institute, 2011), and summarized in Table 2. Furthermore, the susceptibility patterns of *Nocardia* in our study were compared with those described in previous studies (Larruskain et al., 2011; McTaggart et al., 2015).

2.4. Statistical analysis

MIC_{50} and MIC_{90} are defined as the MICs of a given agent that inhibits the growth of 50% and 90% of the isolates, respectively. The MIC data of each antibiotic were recorded and analyzed by WHONET 5.6 software, and MIC_{50} and MIC_{90} were also calculated. Furthermore, the distribution of *Nocardia* species as well as ages and infection types was determined by using GraphPad Prism version 6.02.

3. Results

3.1. Demographic data of 53 Nocardia isolates

A total of 53 *Nocardia* isolates, recovered from 53 patients respectively, were identified during 2009 and 2017 from 8 hospitals of 7 cities in China, with demographic and clinical characteristics summarized in Table 1. There are 34 *Nocardia* isolate (64.2%) collected from sputum, BALF and pleural effusion), and 14 isolates (26.4%) were recovered from skin and soft tissue wound, pus, lesion and abscess, two from CSF (*N. asiatica* and *N. nova*), one from bone marrow, one from brain abscess and one from synovial fluid, respectively. The enrolled patients aged from 15 to 93 years (with the median age of 60 years), and 62.3% (33/53) ≥ 60 years and only 13.2% (7/53) less than 40 years, as shown in Fig. 1. No disseminated infection was detected in patients less than 40 years, 85.7% (6/7) of whom suffered superficial infection. The gender ratio of male/female was approximately 1:1 (28/25) with no significant difference. The most common underlying disease was diabetes mellitus (9.4%, 5/53). No nosocomial nocardiosis was identified.

3.2. Distribution of Nocardia species

Among the 53 *Nocardia* isolates, 12 species were confirmed, with the clinical features shown in Table 1 and Fig. 2. *N. farcinica* was the most common species, accounted for 24.5% (13/53), followed by *N. cyriacigeorgica* (20.8%, 11/53), *N. terpenica* (15.1%, 8/53), *N. abscessus* (9.43%, 5/53), *N. otitidiscaviarum* (7.55%, 4/53), *N. nova* (5.66%, 3/53), *N. asiatica* (3.77%, 2/53), *N. blacklockiae* (3.77%, 2/53), *N. brasiliensis* (3.77%, 2/53), *N. beijingensis* (1.89%, 1/53), *N. pseudobrasilliensis* (1.89%, 1/53), and *N. transvalensis* (1.89%, 1/53), respectively.

According to the criteria for judging invasive and noninvasive cases, there were 13 (24.5%) *Nocardia* stains isolated from sterile sites considered to be invasive, and other 40 isolates noninvasive. No significant difference of invasive and noninvasive cases was found between different *Nocardia* species.

3.3. Antibiotic susceptibility profiles

The susceptibility to 10 commonly-used antibiotics for *Nocardia* were summarized in Table 2, showing the MIC_{50}, MIC_{90}, resistance rates for each antibiotic. The antibiotic resistance profiles varied in line with different *Nocardia* species. All of our isolates were linezolid susceptible and most species were also imipenem (90.6%) and amikacin (92.5%) susceptible.

Table 1
Clinical features of 53 *Nocardia* isolates from different regions in China.

Strain no.	Species by MLSA	Specimen type	Gender	Age (y)	Year of isolation	Region (city/province)	Invasive / Noninvasive	Underlying Diseases	Infection type	Clinical diagnosis
FJ-01	*N. terpenica*	skin abscess	male	57	2016	Xiamen, Fujian	invasive	diabetes mellitus	superficial infection	skin abscess
BJ-02	*N. farcinica*	sputum	male	72	2009	Beijing	noninvasive	diabetes mellitus	pulmonary infection	pneumonia
SD-03	*N. nova*	CSF	female	41	2016	Weifang, Shandong	invasive	systemic lupus erythematosus	disseminated infection	brain abscess
SD-04	*N. farcinica*	bone marrow	male	68	2016	Weifang, Shandong	invasive	NA	disseminated infection	osteomyelitis
BJ-05	*N. cyriacigeorgica*	skin abscess	male	93	2016	Beijing	noninvasive	diabetes mellitus	superficial infection	skin abscess
BJ-06	*N. brasiliensis*	skin abscess	male	68	2016	Beijing	noninvasive	diabetes mellitus	superficial infection	skin abscess
GX-07	*N. nova*	sputum	female	83	2015	Nanning, Guangxi	noninvasive	NA	pulmonary infection	pneumonia
GX-08	*N. cyriacigeorgica*	sputum	female	63	2017	Nanning, Guangxi	noninvasive	NA	pulmonary infection	pneumonia
GX-09	*N. farcinica*	sputum	male	59	2016	Nanning, Guangxi	noninvasive	NA	pulmonary infection	pneumonia
GX-10	*N. farcinica*	sputum	female	55	2016	Nanning, Guangxi	noninvasive	NA	pulmonary infection	pneumonia
GX-11	*N. farcinica*	pus	male	58	2016	Nanning, Guangxi	invasive	NA	disseminated infection	brain abscess
GX-12	*N. abscessus*	pus	male	65	2017	Nanning, Guangxi	invasive	NA	superficial infection	neck abscess
GX-13	*N. nova*	pleural effusion	male	62	2017	Nanning, Guangxi	invasive	NA	pulmonary infection	pneumonia/ pleurisy
GX-14	*N. otitidiscaviarium*	sputum	female	60	2017	Nanning, Guangxi	noninvasive	NA	pulmonary infection	pneumonia
GX-15	*N. farcinica*	pleural effusion	male	56	2017	Nanning, Guangxi	invasive	NA	pulmonary infection	pneumonia/ pleurisy
GX-16	*N. otitidiscaviarium*	sputum	male	65	2017	Nanning, Guangxi	noninvasive	NA	pulmonary infection	pneumonia
HN-17	*N. blacklockiae*	sputum	female	61	2017	Liuyang, Hunan	noninvasive	NA	pulmonary infection	pneumonia
HN-18	*N. transvalensis*	sputum	female	73	2017	Liuyang, Hunan	noninvasive	NA	pulmonary infection	pneumonia
BJ-19	*N. farcinica*	pus	male	15	2015	Beijing	invasive	NA	superficial infection	skin abscess
BJ-20	*N. farcinica*	wound	female	21	2014	Beijing	noninvasive	NA	superficial infection	skin lesion
BJ-21	*N. cyriacigeorgica*	sputum	male	89	2015	Beijing	noninvasive	NA	pulmonary infection	pneumonia
BJ-22	*N. cyriacigeorgica*	synovial fluid	male	48	2014	Beijing	invasive	Impaired kidney function	disseminated infection	joint infection
BJ-23	*N. cyriacigeorgica*	BALF	male	60	2015	Beijing	noninvasive	Impaired kidney function	pulmonary infection	pneumonia
BJ-24	*N. farcinica*	sputum	female	72	2010	Beijing	noninvasive	NA	pulmonary infection	pneumonia
BJ-25	*N. cyriacigeorgica*	sputum	male	77	2011	Beijing	noninvasive	NA	pulmonary infection	pneumonia
BJ-26	*N. abscessus*	sputum	female	73	2014	Beijing	noninvasive	NA	pulmonary infection	pneumonia
BJ-27	*N. farcinica*	pleural effusion	male	76	2013	Beijing	invasive	NA	pulmonary infection	pneumonia/ pleurisy
BJ-28	*N. abscessus*	pus	female	18	2014	Beijing	invasive	NA	superficial infection	skin lesion
BJ-29	*N. abscessus*	wound	male	71	2012	Beijing	noninvasive	NA	superficial infection	skin lesion
BJ-30	*N. cyriacigeorgica*	BALF	female	59	2014	Beijing	noninvasive	NA	pulmonary infection	pneumonia
BJ-31	*N. cyriacigeorgica*	BALF	female	53	2015	Beijing	noninvasive	NA	pulmonary infection	pneumonia
BJ-32	*N. cyriacigeorgica*	sputum	female	55	2017	Beijing	noninvasive	NA	pulmonary infection	bronchiectasia/pneumonia
GX-33	*N. cyriacigeorgica*	BALF	male	74	2017	Nanning, Guangxi	noninvasive	NA	pulmonary infection	pneumonia
GX-34	*N. cyriacigeorgica*	sputum	female	68	2017	Nanning, Guangxi	noninvasive	NA	pulmonary infection	pneumonia
BJ-35	*N. farcinica*	sputum	male	58	2017	Beijing	noninvasive	NA	pulmonary infection	pneumonia
BJ-36	*N. farcinica*	sputum	female	62	2017	Beijing	noninvasive	NA	pulmonary infection	pneumonia
CQ-37	*N. asiatica*	sputum	male	68	2017	Chongqing	noninvasive	NA	pulmonary infection	pneumonia
CQ-38	*N. blacklockiae*	sputum	male	71	2017	Chongqing	noninvasive	NA	pulmonary infection	pneumonia
BJ-39	*N. abscessus*	BALF	female	67	2017	Beijing	noninvasive	NA	pulmonary infection	pneumonia
GX-40	*N. asiatica*	CSF	female	62	2017	Nanning, Guangxi	invasive	NA	disseminated infection	brain abscess
HN-41	*N. terpenica*	sputum	female	68	2017	Liuyang, Hunan	noninvasive	NA	pulmonary infection	pneumonia
BJ-42	*N. otitidiscaviarum*	BALF	male	68	2017	Beijing	noninvasive	NA	pulmonary infection	pneumonia
BJ-43	*N. brasiliensis*	pus	male	56	2017	Beijing	invasive	NA	superficial infection	skin lesion
BJ-44	*N. otitidiscaviarum*	sputum	female	71	2017	Beijing	noninvasive	NA	pulmonary infection	pneumonia
FJ-45	*N. terpenica*	wound	male	27	2016	Xiamen, Fujian	noninvasive	NA	superficial infection	drainage, pus
FJ-46	*N. terpenica*	wound	male	61	2016	Xiamen, Fujian	noninvasive	diabetes mellitus	superficial infection	diabetes mellitus foot/skin lesion
FJ-47	*N. terpenica*	wound	male	57	2016	Xiamen, Fujian	noninvasive	NA	superficial infection	skin lesion
FJ-48	*N. terpenica*	wound	female	31	2017	Xiamen, Fujian	noninvasive	NA	superficial infection	skin lesion
FJ-49	*N. farcinica*	sputum	female	71	2017	Xiamen, Fujian	noninvasive	NA	pulmonary infection	pneumonia
FJ-50	*N. pseudobrasilliensis*	sputum	female	35	2017	Xiamen, Fujian	noninvasive	NA	pulmonary infection	pneumonia
FJ-51	*N. beijingensis*	BALF	male	66	2017	Xiamen, Fujian	noninvasive	NA	pulmonary infection	pneumonia
FJ-52	*N. terpenica*	sputum	female	72	2017	Xiamen, Fujian	noninvasive	NA	pulmonary infection	pneumonia
FJ-53	*N. terpenica*	wound, drainage, pus	female	22	2017	Xiamen, Fujian	noninvasive	NA	superficial infection	skin lesion

MLSA = multilocus sequence analysis; CSF = cerebrospinal fluid; BALF = bronchoalveolar lavage fluid; NA = not available.

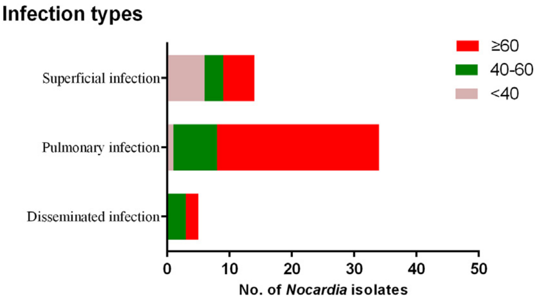

Fig. 1. Correlation between ages and infection types caused by *Nocardia*.

The resistance rates of *N. farcinica* to TMP-SMX (53.8%, 7/13), gentamicin (84.6%, 11/13), cefepime (69.2%, 9/13), and ceftriaxone (69.2%, 9/13) were relatively high. In addition, the resistance rate of *N. cyriacigeorgica* to ciprofloxacin (63.6%, 7/11) was high, too. Similarly, 87.5%(7/8) *N. terpenica* was resistant to amoxicillin-clavulanate and 25.0% showed TMP-SMX resistant (Table 2).

3.4. Correlation of antimicrobial susceptibility profiles with Nocardia species designation

The antimicrobial susceptibility pattern types in the present study were compared with the reports by Larruskain et al. (2011) and McTaggart et al. (2015). A strong correlation between the drug pattern types and *Nocardia* species identification was identified and demonstrated in Table 3. However, some discrepancies were noted, and of particular interest are the *N. abscesuss* isolates, type I pattern, 100% of which were susceptible to imipenem. There were 8 *N. terpenica* strains, mainly (87.5%, 7/8) collected from Xiamen city in current study, having a different drug susceptibility profile (Table 3).

3.5. Correlation of antimicrobial susceptibility profiles with isolation geography and time

Among various regions, the antibiotic resistance rates of Weifang, Shandong province were lower than other regions. And no significant relationship between geography and resistance rate profile from other regions was found. Furthermore,- the number of collected *Nocardia* isolates increased steadily from 2015 to 2017, the resistance rates of various antibiotics changed unevenly between various years, and no significant variation was found. The antibiotic resistance rates of *N. farcinica*, collected mainly from Fujian province, were significantly higher than other *Nocardia* species, as summarized in Tables 2 and 4.

4. Discussion

The opportunistic infections by *Nocardia* species, an atypical aerobic pathogen, usually affects the elderly and/or those with chronic diseases or under immunocompromised conditions, and occasionally immunocompetent hosts, *via* direct inoculation through the skin or by inhalation (Guerra et al., 2015; Wang et al., 2015; Wilson, 2012). In the current study, the nocardiosis patients were in similar conditions: old age (averaged 60 years) and immunocompromised, including diabetes mellitus. Furthermore, in line with our data, the patients aged less than 40 years might have more chances for superficial infection rather than respiratory and disseminated infection. Invasive cases were far fewer than noninvasive cases, mainly due to the specimens from respiratory tract accounting for a large percentage (64.2%).

More than 50 species of *Nocardia* have been identified to be human pathogens (Wilson, 2012), and these species distributed differently between various geographic regions. Currently only a few species (*N. brasiliensis*, *N. farcinica*, and *N. pseudobrasiliensis*) can be reliably identified by traditional biochemical methods (Brown-Elliott et al., 2006). For other *Nocardia* species, McTaggart and colleagues documented that a MLSA of the 16S rRNA, secA1, *hsp*65, *rpo*B and *gyr*B genes, will identify them to species level (Baio et al., 2013; Conville et al., 2006; Takeda et al., 2010; Yin et al., 2007). In line with MLSA results, in our study, 12 *Nocardia* species were identified, of which *N. farcinica* was the most frequently isolated. Furthermore, *N. farcinica* and *N. cyriacigeorgica* were the most common species in Beijing, which was similar to Xiao *et al*’ report from Beijing (Xiao et al., 2016), and 7 out 8 *N. terpenica* isolates were identified from Xiamen, Fujian Province, 4 out of 5 *N. abscessus* from Beijing, hinting the species distribution of *Nocardia* might be geographically-related. The distributional features herein were different from United States (Uhde et al., 2010), Taiwan (Wang et al., 2015), Spain (Valdezate et al., 2015, 2017), and Iran (Hashemi-Shahraki et al., 2015), where *N. nova*, *N. brasiliensis*, *N. asteroids*, and *N. asteroids* were the most predominant species, respectively. In addition, in accordance with our data, it was worth mentioning that *N. terpenica* mainly caused superficial infections (6 isolates, skin abscess/lesions) and lower respiratory tract infections (2 isolates). This species has been documented to be collected from respiratory tracts of two patients in Japan, as the closest members of *N. nova* (Hoshino et al., 2007). Therefore, our observations will broaden the knowledge of the pathogenesis of *N. terpenica* as a causative agent. Jiang Y *et al* have reviewed 23 clinical infection cases caused by *N. otitidiscaviarum*, only 5 patients with pulmonary involvement (Jiang et al., 2016). However, all 4 *N. otitidiscaviarum* isolates in our study were identified from patients with pneumonia, as might be explained by geographical differences.

Furthermore, the antimicrobial susceptibility patterns of the well-recognized species listed for comparison in Tables 2 and 3. The

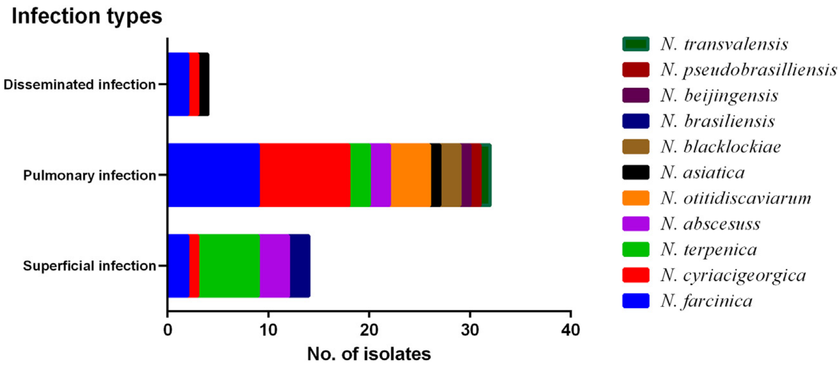

Fig. 2. *Nocardia* species distribution grouped by infection types.

Table 2
Antimicrobial susceptibility and MICs of 53 *Nocardia* isolates in China.

Antibiotics / Different species	Breakpoints	Species (no. of strains, %)					
		All *Nocardia* 53 (100%)	*N. farcinica* 13 (24.5%)	*N. cyriacigeorgica* 11 (20.8%)	*N. terpenica* 8 (15.1%)	*N. abscesuss* 5 (9.43%)	*N. otitidiscaviarum* 4 (7.55%)
AMC	S ≤ 8/4, R ≥ 32/16						
MIC$_{50}$ (mg/L)		4	1.5	4	256	0.19	256
MIC$_{90}$ (mg/L)		256	2	64	256	0.38	256
resistance, n (%)		18 (34.0%)	0 (0%)	2 (18.2%)	7 (87.5%)	0 (0%)	4 (100%)
ciprofloxacin	S ≤ 1, R ≥ 4						
MIC$_{50}$ (mg/L)		1.5	0.75	4	0.75	6	1
MIC$_{90}$ (mg/L)		32	3	32	2	32	8
resistance, n (%)		18 (34.0%)	2 (15.4%)	7 (63.6%)	0 (0%)	3 (60%)	2 (50%)
ceftriaxone	S ≤ 8, R ≥ 64						
MIC$_{50}$ (mg/L)		1	32	0.38	0.5	0.19	32
MIC$_{90}$ (mg/L)		32	32	2	32	0.5	256
resistance, n (%)		16 (30.2%)	7 (53.8%)	0 (0%)	2 (25%)	0 (0%)	4 (100%)
imipenem	S ≤ 4, R ≥ 16						
MIC$_{50}$ (mg/L)		0.38	0.75	0.25	0.032	0.19	16
MIC$_{90}$ (mg/L)		4	1.5	1	0.38	2	32
resistance, n (%)		5 (9.4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (75%)
linezolid	S ≤ 8						
MIC$_{50}$ (mg/L)		1	1.5	0.38	0.5	0.25	0.38
MIC$_{90}$ (mg/L)		6	8	1.5	8	1	2
resistance, n (%)		0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
TMP-SMX	S ≤ 2/38, R ≥ 4/76						
MIC$_{50}$ (mg/L)		0.75	6	0.25	2	0.25	2
MIC$_{90}$ (mg/L)		32	32	0.75	32	0.75	2
resistance, n (%)		14 (26.4%)	7 (53.8%)	1 (9.1%)	2 (25%)	0 (0%)	0 (0%)
amikacin	S ≤ 8, R ≥ 16						
MIC$_{50}$ (mg/L)		0.5	1	0.5	0.38	0.19	0.25
MIC$_{90}$ (mg/L)		8	1.5	1.5	12	0.38	0.75
resistance, n (%)		4 (7.5%)	1 (7.7%)	0 (0%)	1 (12.5%)	0 (0%)	0 (0%)
gentamicin	S ≤ 4, R ≥ 16						
MIC$_{50}$ (mg/L)		0.25	48	0.25	0.064	0.064	0.023
MIC$_{90}$ (mg/L)		96	128	0.38	0.19	0.25	0.25
resistance, n (%)		12 (22.6%)	11 (84.6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
cefepime	S ≤ 8, R ≥ 32						
MIC$_{50}$ (mg/L)		1	64	0.5	0.5	0.125	256
MIC$_{90}$ (mg/L)		256	256	2	4	0.25	256
resistance, n (%)		14 (26.4%)	9 (69.2%)	0 (0%)	0 (0%)	0 (0%)	4 (100%)
cefotaxime	S ≤ 8, R ≥ 64						
MIC$_{50}$ (mg/L)		0.5	256	0.19	0.125	0.064	256
MIC$_{90}$ (mg/L)		256	256	0.5	1	0.094	256
resistance, n (%)		13 (24.5%)	9 (69.2%)	0 (0%)	0 (0%)	0 (0%)	4 (100%)

AMC = amoxicillin-clavulanate; TMP-SMX = Trimethoprim-sulfamethoxazole. Other *Nocardia* species, such as *N. nova*, *N. asiatica*, *N. blacklockiae*, *N. brasiliensis* et al., were ≤3 isolates. They were not suitable to calculate MIC$_{50}$, MIC$_{90}$, and resistance rates, thus not included in the table.

susceptibility test should be performed when patients present with deep-seated nocardiosis with cerebral involvement, and relapse or disseminated infections, or the relatively resistant causative species (mainly *N. farcinica*) was identified. To date, TMP-SMX constitutes the mainstay of antimicrobial therapy for nocardiosis (Valdezate et al., 2015), while its resistance rates to *Nocardia* varied greatly among different regions of the world, as reported to be 16.2%(181/1119), 42%(321/765), and 0% in Spain, the United States and Belgian, respectively (Glupczynski et al., 2006; Uhde et al., 2010; Valdezate et al., 2017). Cercenado et al. (2007) and Torres et al. (2000) found 18% and 53% TMP-SMX resistance in *N. farcinica* isolates, respectively. Our results showed the resistance rate of TMP-SMX was 26.4% to all *Nocardia* species, majority of which were *N. farcinica* (53.8%). Although TMP-SMX is the choice of treatment for nocariosis, other suitable regimen might be considered due to the occurrence of sulfonamide drug allergies and resistance. The beta-lactam antibiotics were sometimes used as alternative to TMP-SMX. Like the findings in previous studies (Hashemi-Shahraki et al., 2015; McTaggart et al., 2015), the *Nocardia* species remained good susceptibility to imipenem, only 5 isolates (3 *N. otitidiscaviarum* and 2 *N. brasiliensis*) were imipenem-resistant in accordance with our data. Furthermore, our isolates showed various susceptibilities to other beta-lactam antibiotics, including amoxicillin-clavlautic acid (34.0% resistance rate) and ceftriaxone (30.2%), indicating an AST should be conducted if necessary. Amikacin has been used successfully, usually in combination with other agents, in patients with nocardiosis involving the immunocompromised or aging patients. The agent showed a good susceptibility against a variety of *Nocardia* species, only 4(7.5%) amikacin resistant isolates were identified in current study, as similar to previous reports (Hashemi-Shahraki et al., 2015). The most recent therapeutic alternative is linezolid, having 100% sensitivity to *Nocardia* species *in vitro* in the current study. Consequently, linezolid, imipenem and amikacin could be potentially used for empiric treatment of nocardiosis in China. But the accurate identification and susceptibility testing is recommended for patients with systemic diseases to guide combination therapy, as the susceptibility varied among different *Nocardia* species. Because of the scarce experience of fluoroquinolones in nocardiosis, these drugs, if tested susceptible, will probably remain as alternatives when the first-line antimicrobial agents cannot be used or is resistant. In current study, ciprofloxacin showed a species-specific susceptibility: all *N. terpenica* isolates were susceptible or intermediate to the agent, while 15.4% of *N. farcinica*, 63.3% of *N. cyriacigeorgica* and 50% *N. otitidiscaviarium* were resistant. For many of the remaining antimicrobials, substantial numbers of *Nocardia* isolates were resistant, and resistance was often species specific.

As reported previously, *Nocardia* taxonomy has been linked to specific patterns of antimicrobial susceptibility (McTaggart et al., 2015). Among the top 5 frequently isolated *Nocardia* species in our study, the

Table 3
Antimicrobial susceptibility patterns with *Nocardia* species designation.

Nocardia Species	No. of isolates in the study	Drug Patterns types	Antimicrobial susceptibility pattern defined by Brown-Elliot [2,20] or McTaggart [9]							
			Resistant or non-susceptible (intermediate and resistant)				Susceptible			
			Antibiotics	Larruskain *et al.* [2]* (%)	McTaggart *et al.* [9] (%)	This study (%)*	Antibiotics	Larruskain *et al.* [20]	McTaggart *et al.*	This study (%)
N. abscesuss	5	I	Ciprofloxacin	100	89	60(100)	AMC	91.3	≥90	100
			Imipenem	39.1(69.6)	78	0	Amikacin	100	≥90	100
							Ceftriaxone	100	≥90	100
							Linezolid	100	≥90	100
							TMP-SMX	NA	≥90	100
							Cefepime	NA	≥90	100
N. nova	3	III	AMC	89.1(92.7)	74	33.3	Linezolid	100	≥90	100
			Ciprofloxacin	NA	≥90	66.7	Ceftriaxone	74.5	85	33.3
							Imipenem	98.2	≥90	100
							Amikacin	100	≥90	66.7
							TMP-SMX	NA	≥90	100
							Cefepime	NA	74	100
								NA	≥90	66.7
N. transvalensis complex	1	IV	Imipenem	NA	≥90	0	Ceftriaxone	NA	≥90	100
			Amikacin	NA	80	100	Ciprofloxacin	NA	≥90	100
							Linezolid	NA	≥90	100
							TMP-SMX	NA	80	0
N. farcinica	13	V	Ceftriaxone	51.2(100)	≥90	53.8(61.5)	Amikacin	100	≥90	92.3
			Gentamicin	100	NA		Ciprofloxacin(variable Susceptibility)	18.6	50	69.2
			Cefepime	NA	≥90	76.9(84.6)				
						69.2	Imipenem(variable susceptibility)	72.1	53	100
							Linezolid	100	≥90	100
							AMC	NA	78	100
							TMP-SMX	NA	≥90	46.2
N. cyriacigeorgica	11	VI	AMC	96.4(100)	80	18.2	Amikacin	100	≥90	100
			Ciprofloxacin	78.6(100)	≥90	63.6(81.8)	Ceftriaxone	92.9	≥90	100
							Imipenem	89.3	≥90	100
							Linezolid	100	≥90	100
							TMP-SMX	NA	≥90	91.9
N. otitidiscaviarum	4	VII	Ceftriaxone	NA	≥90	100	Amikacin	NA	≥90	100
			AMC	NA	≥90	100	Linezolid	NA	≥90	100
			Imipenem	NA	≥90	75	TMP-SMX	NA	≥90	100
			Cefepime	NA	≥90	100				
			Ciprofloxacin	NA	≥90	50				
N. brasiliensis	2	VIII	Cefepime	NA	≥90	50	AMC	NA	≥90	100
			Imipenem	NA	83	100	Amikacin	NA	≥90	100
			Ciprofloxacin	NA	≥90	50(100)	Linezolid	NA	≥90	100
							TMP-SMX	NA	≥90	100
N. terpenica	8	NA	AMC	NA	NA	87.5	Imipenem	NA	NA	100
							Ciprofloxacin	NA	NA	75
							Linezolid	NA	NA	100
							TMP-SMX	NA	NA	75
							Amikacin	NA	NA	87.5
							Cefepime	NA	NA	100

AMC = amoxicillin-clavulanate; TMP-SMX = Trimethoprim-sulfamethoxazolel; NA = Not available. *Values in parentheses represents the percentage of *Nocardia* isolates (with resistant and intermediate susceptibilities).

Table 4
Relationship between antibiotic resistance profile and geography as well as isolation time.

Isolation location and time	Total No. of isolates	Antibiotic resistance rates								
		amikacin	TMP-SMX	gentamicin	ciprofloxacin	cefotaxime	cefepime	imipenem	ceftriaxone	AMC
Geography										
Beijing	23	0%	21.7%	21.7%	52.2%	26.1%	30.4%	8.7%	21.7%	17.4%
Nanning and Liuzhou, Guangxi	13	0%	30.8%	30.8%	38.5%	46.1%	46.1%	15.4%	61.5%	30.8%
Xiamen, Fujian	10	10.0%	20.0%	20.0%	10.0%	10.0%	10.0%	10.0%	30.0%	70.0%
Liuyang, Hunan	3	33.3%	66.7%	0%	0%	0%	0%	0%	0%	66.7%
Weifang, Shandong	2	100%	0%	0%	0%	0%	0%	0%	0%	0%
Chongqing	2	0%	50.0%	50.0%	0%	0%	0%	0%	0%	50.0%
Isolation year										
2017	27	8.3%	25.9%	22.2%	33.3%	22.2%	29.6%	15.0%	25.9%	44.4%
2016	11	18.2%	27.3%	27.3%	0%	27.3%	27.3%	9.1%	45.5%	36.4%
2015	5	0%	20.0%	20.0%	80.0%	20.0%	20.0%	0%	40.0%	40.0%
2014	5	0%	20.0%	0%	40.0%	0%	0%	0%	0%	0%
2009–2013	5	0%	40.0%	40.0%	60.0%	40.0%	40.0%	0%	40.0%	0%

AMC = amoxicillin-clavulanate; TMP-SMX = trimethoprim-sulfamethoxazole.

resistance rates of *N. farcinica* were the highest. Susceptibility patterns themselves might be unnecessarily indicative of a particular species, but if associated with other phenotypic characteristics, they can hint classification within a *Nocardia* species or group. As previously described by Brown-Elliott et al. (2006) and other studies (Glupczynski et al., 2006; Hashemi-Shahraki et al., 2015; Larruskain et al., 2011; McTaggart et al., 2015; Tremblay et al., 2011), we also noted a strong correlation between the drug pattern types and species in our data. However, some discrepancies were also noted. In the present study, no isolates of *N. brevicatena/paucivorans* (type II drug pattern) were identified, as similar with McTaggart et al. (2015). In particular, Brown-Elliott et al. (2006) reported that the *N. transvalensis* complex was susceptible to imipenem, but McTaggart et al. (2015) noted imipenem-resistance among all isolates. In our study only one *N. transvalensis* isolate was detected and imipenem-susceptible. With respect to *N. farcinica*, Brown-Elliott et al. (2006) indicated that it was susceptible to imipenem and ciprofloxacin, while McTaggart et al. (2015) noted susceptibility only among approximately half of the isolates. Our results was similar to Brown-Elliott's, with 84.6% (9/13) and 100% isolates susceptible to ciprofloxacin and imipenem, respectively. Moreover, unlike Brown-Elliott et al. (2006), who indicated that *N. otitidiscaviarum* isolates were susceptible to ciprofloxacin, McTaggart *et al* reported all were resistant (McTaggart et al., 2015). In our report, half isolates of *N. otitidiscaviarum* were susceptible. The above discrepancies may be explained by different geographic isolation and the long-term evolution of drug resistance. In addition to the antimicrobial susceptibility patterns of these well-recognized species (Larruskain et al., 2011; McTaggart et al., 2015), we also report preliminary antimicrobial susceptibility patterns for isolates of species that were not categorized within these traditionally acknowledged groups and yet were recovered from clinical specimens, *N. terpenica*. This *Nocardia* species, accounting for 8 isolates in our study, was mainly recovered from Xiamen city, and rarely reported elsewhere (Hoshino et al., 2007). Therefore, it is categorized as a new group and listed in the Table 3. *N. terpenica* isolates exhibited a susceptibility pattern similar to that of the type III defined by McTaggart et al. (2015). However, most *N. terpenica* isolates (75% and 25%, respectively) were susceptible and intermediate to ciprofloxacin, differing from type III drug pattern (Table 3). Potentially, these could comprise a new group, if susceptibility testing profiles of future additional isolates support the novel pattern. Taken together, species identification for *Nocardia* is predictive of antimicrobial susceptibility (Hashemi-Shahraki et al., 2015; McTaggart et al., 2015). If the susceptibility testing results were contrary to the well-recognized patterns, they should be explained with caution and confirmed.

Moreover, some *Nocardia* species were rarely recovered from clinical specimens, and could not be grouped within the traditional categories. This study also presented their antimicrobial susceptibility for isolates of species. Collectively these isolates represent approximately 11.3%, and some of them lack published MIC data. It is not surprising of the emergency of uncommon antibiotic resistance pattern of *Nocardia* species due to the extended treatment strategy for nocardiosis. Our results will be useful for the treatment regimen in patients with nocardiosis in China and worldwide.

Our study is obviously limited by several factors. First, the sample size is relatively small within the study time periods, and the epidemiological features might only stand for limited regions involved, therefore reducing the robustness of the distributional characteristics and antibiograms for some species, thus further studies with larger sample size are still needed to confirm our conclusion. Also, due to the small sample size, we could not get the accurate statistical relationship between geography and antibiotic resistance profile. Second, E-test stripe test was performed in previous studies for *Nocardia* spp. (Ambaye et al., 1997; Glupczynski et al., 2006; Valdezate et al., 2017), and easy to use in the routine clinical laboratory; however, discrepancies between laboratories have been documented due to inherent technical difficulties in end-point determination (Lowman and Aithma, 2010). The lack of reproducibility of the broth microdilution method for *Nocardia* species has also been documented due to the inherent slow growth characteristics of the species. Put together, the susceptibility testing result is a helpful guide for nocardiosis treatment, but it should be interpreted with caution. The data were presented only as a preliminary step in assessing empirical treatment options for infections caused by both the common and rarely encountered species of *Nocardia* in mainland China. The national surveillance system for nocardiosis is urgently required to longitudinally monitor the distribution and the *in vitro* antimicrobial susceptibility profiles of *Nocardia* isolates.

In conclusion, the present study conducted a retrospective review of 53 non-repetitive clinical *Nocardia* species from 7 cities in China, and the distribution and antibiotic profiles of the identified *Nocardia* species were characterized. Our results will contribute to better understand the clinical features of nocardiosis and *Nocardia* species distributed in China and for decision-making in the context of empirical treatment.

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Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval

This study was approved by the ethics committee of Peking University First Hospital, in Beijing, China.

Informed consent

The benefits and privacy of involved patients were not affected, and informed consents were waived.

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