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VETERINARY MICROBIOLOGY - RESEARCH PAPER

Molecular identification and antimicrobial resistance pattern of *Nocardia* isolated from 14 diseased dogs and cats

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Abstract

Nocardia are ubiquitous, saprophytic and opportunistic bacteria. They cause a set of pyogenic clinical infections in animals and humans, particularly immunocompromised patients, mostly affecting the skin and respiratory tract, with refractoriness to conventional therapy. The most descriptions of nocardial infections in companion animals involve case reports, and there are scarce case series studies focused on canine and feline nocardiosis in which diagnosis has been based on molecular techniques. We investigated epidemiological aspects, clinical findings, in vitro susceptibility profile, and molecular identification of *Nocardia* using PCR-based method targeted 16S rRNA gene in twelve dogs and two cats. Among dogs were observed cutaneous lesions (8/12 = 67%), pneumonia (3/12 = 25%), and encephalitis (2/12 = 17%), whereas cats developed cutaneous lesions and osteomyelitis. *Nocardia* and canine morbillivirus coinfection was described in six dogs (6/12 = 50%). A high mortality rate (6/8 = 75%) was seen among dogs. Three dogs (3/4 = 75%) and one cat (1/2 = 50%) with systemic signs (pneumonia, encephalitis, osteomyelitis), and 83% (5/6) of dogs with a history of concomitant morbillivirus infection died. *N. nova* (5/12 = 42%), *N. cyriacigeorgica* (3/12 = 25%), *N. farcinica* (2/12 = 17%), *N. veterana* (1/12 = 8%), and *N. asteroides* (1/12 = 8%) species were identified in dogs, whereas *N. africana* and *N. veterana* in cats. Among the isolates from dogs, cefuroxime (12/12 = 100%), amikacin (10/12 = 83%), gentamycin (10/12 = 83%), and imipenem (10/12 = 83%) were the most effective antimicrobials, whereas cefuroxime, cephalexin, amoxicillin/clavulanic acid, imipenem, and gentamycin were efficient against isolates from cats. Multidrug resistance was observed in 36% (5/14) of isolates. We describe a variety of *Nocardia* species infecting dogs and cats, multidrug-resistant ones, and a high mortality rate, highlighting a poor prognosis of nocardiosis in companion animals, particularly among animals systemically compromised or coinfected by canine morbillivirus. Our study contributes to species identification, in vitro antimicrobial susceptibility profile, clinical-epidemiological aspects, and outcome of natural *Nocardia*-acquired infections in dogs and cats.

Keywords Canine and feline nocardiosis · Comorbidity · Molecular identification · Antimicrobial profile

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Introduction

Nocardia is a complex group of facultative intracellular bacteria, opportunistic in nature, which can affect domestic animals, wildlife, and humans [1]. These aerobic actinomycetes typically appear as long or branching gram-positive filamentous microorganisms, whose colonies present a powdery surface that resembles fungal organisms [2]. *Nocardia* species are ubiquitous saprophytic microorganisms widely distributed in soil, degraded organic and plant matter, water, dust, and other environmental sources [3].

The *Nocardia asteroides* complex (*N. asteroides*, *N. nova, N. farcinica*), as well as *N. brasiliensis*, *N. pseudobrasiliensis*, *N. otitidiscaviarum*, and *N. transvalensis* were the main species described in diseased domestic animals and humans based on former phenotypic classification [4]. In the last decades, PCR-based methods, and sequencing of 16S rRNA have enabled taxonomic reclassification and inclusion of new species of *Nocardia* [1, 5–7]. To date, 119 species of *Nocardia* are taxonomically known with validly published names on the "List of Prokaryotic names with Standing Nomenclature" [8], although it is estimated that approximately 50 species are pathogenic to humans [1] and/or animals [3].

N. otitidiscaviarum, *N. cyriacigeorgica*, *N. nova*, and *N. farcinica* have been currently reported in diseased domestic animals based on molecular speciation [4], with some geographic variation of pathogenic species among different countries [9, 10]. Particularly in dogs, *N. abscessus*, *N. cyriacigeorgica, N. asiatica*, *N. pseudobrasiliensis*, and *N. veterana* have been reported [11–15], whereas *N. africana*, *N. jiangxiensis*, and *N. tenerifensis* have been identified in cats [16–18].

Traumatic inoculation of the pathogen through punctures, foreign bodies, inhalation, and bite or scratches injuries appear to be the main routes of *Nocardia* transmission among dogs and cats [14, 17, 19]. The disease is considered uncommon or rare in companion animals [4, 9], with most descriptions restricted to case reports in dogs [11–15] and cats [16–18].

Cutaneous-subcutaneous lesions [16], pneumonia [15], and systemic infections (e.g., osteomyelitis, peritonitis, pleural effusion, organ abscesses, and encephalitis) are the most frequent clinical forms in companion animals [9, 12, 13, 17, 19]. Moreover, most of the described cases are related to underlying conditions or immunosuppressive disorders, particularly dogs coinfected with canine (distemper) morbillivirus [15], and cats positive for immunodeficiency and leukemia virus [9].

Routine laboratory diagnosis of nocardial infections in domestic animals has been based on microbiological culture and phenotypic (biochemical) tests, including carbohydrate assimilation and hydrolysis of different substrates [12, 19]. However, some of these tests are time-consuming and not accurate enough to differentiate the pathogen at the species-level. Also, due to the great variety of *Nocardia* species, the molecular-based diagnosis has enabled to distinguish among the clinically relevant species [1, 5–7], including from companion animals [11–14, 17].

Nocardial infections in companion animals are commonly refractory to conventional antimicrobial treatments, with better outcomes in cutaneous infections [4, 19]. Long-term therapy is required, usually resulting in a guarded prognosis as observed in systemic infections, especially among animals with underlying conditions or coinfected with immunosuppressive pathogens [9, 12, 13, 15, 17].

In human patients, an increasing number of *Nocardia* infections has been reported, particularly among immunocompromised people [20]. In addition, nocardiosis has been considered a neglected disease due to its broad spectrum of clinical presentations and similarities with other infectious diseases [6, 7]. The clear impact of pets-to-humans transmission of *Nocardia* is poorly understood, although the identification of well-known pathogenic species of *Nocardia* in diseased companion animals is relevant in human health [4]. Considering this scenario, we investigated selected epidemiological data, clinical findings, in vitro susceptibility pattern, and molecular speciation of *Nocardia* isolates based on the 16S rRNA gene, among twelve diseased dogs and two cats.

Materials and methods

Study design

A cross-sectional study of selected epidemiological variables, clinical features, in vitro susceptibility patterns, and molecular speciation of *Nocardia* isolated from 14 diseased companion animals case series was investigated from 2001 to 2010.

Animals

A convenience sample of 12 dogs and 2 cats with the diagnosis of nocardiosis from three Teaching Veterinary Hospitals (São Paulo State University-UNESP, Botucatu, SP; Federal University of Santa Maria, RS; and Pontifical Catholic University of Paraná, Curitiba, PR) located in the South and Southeast regions from Brazil were included in this study.

Epidemiological and clinical data

The selected epidemiological data investigated were gender (male/female), age, date (year), predisposing factors/comorbidities, and outcome (survival or death). Age of dogs

and cats were stratified as follows: < 1-year-old (puppy), 1- to < 5-years-old (young), 5- to ≤ 10-years-old (adult), and > 10-years-old (senior).

The diagnosis was based on clinical examination, complementary exams, microbiological culture, and molecular identification of *Nocardia* isolates based on the 16S rRNA gene. Clinical specimens of dogs and cats were aseptically collected from cutaneous-subcutaneous abscesses, bronchoalveolar lavage, cerebrospinal fluid, aspirates from lymph nodes, and discharge from osteochondral lesions.

Bacteriological diagnosis

All the clinical samples were plated on defibrinated sheep blood agar (5%) and MacConkey agar (Oxoid®, São Paulo, Brazil), incubated aerobically at 37 °C for 72 h [2]. Simultaneously, all samples were plated on Dextrose Sabouraud agar (Oxoid®, São Paulo, Brazil) and maintained in aerobiosis at 37 °C for 15 days [21]. Dry, circular, convex, firmly adherent to medium, with a powdery surface, and whitish-to-cream colonies suggestive of *Nocardia* species were subjected to Gram and Kinyoun staining (modified Ziehl Neelsen—MZN) [2]. Gram-positive, filamentous, partially acid-fast organisms were presumptively identified as *Nocardia* sp.[2, 21], and stored for further molecular analysis.

In vitro antimicrobial susceptibility test

Nocardia isolates were subjected to in vitro antimicrobial disk diffusion test based on Clinical and Laboratory Standards Institute-CLSI [22] guidelines, with some modifications, using ten antimicrobials from three different classes, as follows: (1) aminoglycosides (amikacin 30 µg, gentamicin 10 µg), (2) beta-lactams (ampicillin 10 µg, amoxicillin/clavulanic acid 30 µg, imipenem 10 µg, ceftiofur 30 µg, cefuroxime 30 µg, ceftriaxone 30 µg, cephalexin 30 µg), and (3) potentiated sulfonamides (trimethoprim-sulfamethoxazole 25 µg). In brief, all isolates were cultured twice on sheep blood agar (5%) to ensure purity. After 48 h, the isolates were inoculated in brain heart infusion broth and incubated at 35 ± 2 °C for 48 h. Sterile glass beads were added to decrease clump formation, a known characteristic in actinomycetes growth. Then, the suspensions were gently vortexed until the appropriate optical density (OD) of 0.5 in the McFarland scale to inoculate the recommended amount of colony-forming unit (CFU). Inhibition zones and classification of isolates as susceptible, intermediate, and resistant were interpreted after 48–72 h of incubation, according to previous studies [10, 23].

Isolates that exhibited simultaneous resistance to ≥ 3 different classes of antimicrobials were considered multidrugresistant [24].

Cytological and histological examination

Imprint and fine needle aspiration cytology from cerebrospinal fluid (2 cases in dogs) and cutaneous lesions (a total of 8 cases, 6 from dogs and 2 from cats) were subjected to cytology (Gram and Diff-Quick staining). Histological examination was performed in 3 cases involving cutaneous lesions, and osteomyelitis infection in 2 cats and 1 dog (H&E, Grocott-Gomori, and PAS staining).

Molecular identification

The diagnosis of species-level among 14 *Nocardia* isolates was performed using a PCR-based 16S rRNA gene, along with phylogenetic analysis [25, 26]. Briefly, the broth culture of each isolate was centrifuged at 12.000 rpm, and the subsequent pellet was resuspended in 200 µL buffer, 250 µL GPT reagent, and 450 µL Tris-buffered phenol (pH 8.0). The tube was placed in the water bath at boiling temperature for 15 min and extract afterwards with 250 µl chloroform/isoamyl alcohol (24:1, v/v). The solution was centrifuged at 12,000 rpm, and the aqueous phase was mixed with 500 µL 100% 2-propanol and 50 µL 3 M sodium acetate. The tube was again centrifuged at 12,000 rpm for 15 min at 4° C and the supernatant was decanted. A final pellet was resuspended in 50 µl TE buffer, after the removal of extraction reagents.

Sequencing of 16 rDNA was amplified using 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 691R (5′-ACCGCTACACCAGGA-3′), 520F (5′-CAGCAGCCGCGGTAATAC-3′) and 1100R (5′-GGGTTGCGCTG TTG-3′), and 926F (5′-AAACTCAAAGGAATTGACGG-3′) and 1542R primers (5′-ACAAAGGAGGTGATC-3′). PCR was performed with DNA thermal cycler using the following conditions: 35 cycles of denaturation at 94 °C for 60 s, annealing at 60 °C for 60 s, and extension at 72 °C for 120 s. The PCR products were purified with a CentriSep Column (Princeton Separations™, Adelphia, NJ, USA). The DNA sequences were determined using the dye terminator cycle sequencing kit (PE Applied Biosystems™) through an automatic sequence analyzer (ABI PRISM 310™, PE Applied Biosystems, Foster City, CA, USA). The species were identified using the sequences of the 16S rDNA segments (1491 bp) based on its 99.6% or higher sequence similarity to the reference sequence in GenBank (DDBJ/GenBank/EMBL) belonging BLAST.

Data analysis

Epidemiological data, clinical findings, *Nocardia* species, and antimicrobial susceptibility patterns were described in absolute numbers and respective percentages. Inference

analysis was not conducted due to restrictions in data availability for each case, which may be considered a limitation in this study.

Results

Microbiological culture

Different clinical specimens from companion animals cultured revealed on blood and Dextrose Sabouraud agar, circular, convex, smooth-to-rough, odorless, non-hemolytic, firmly adherent colonies, displaying various pigmentations (white, cream, and orange) compatible with *Nocardia* species.

In vitro antimicrobial susceptibility pattern

Cefuroxime (12/12 = 100%), amikacin (10/12 = 83%), gentamycin (10/12 = 8%), and imipenem (10/12 = 83%) were the most effective antimicrobials against the isolates from dogs, whereas cefuroxime, cephalexin, amoxicillin/clavulanic acid, imipenem, and gentamycin were 100% efficient against the two isolates from cats. In turn, the most common resistance of the isolates recovered from dogs was seen to ampicillin (7/12 = 58%) and cephalexin (3/12 = 25%) (Table 1). Multidrug resistance isolates to ≥ 3 classes of drugs were found in 36% (5/14) of *Nocardia* species.

Epidemiological and clinical data

Epidemiological data, clinical signs, and molecular speciation of 14 companion animals infected by *Nocardia* are summarized in Table 2.

Among the 12 dogs enrolled, 42% (5/12) were male, 25% (3/12) female, and there was no information or missing data regarding the gender of 33% (4/12) of the animals, while one cat was male and another female. Canine nocardiosis occurred predominantly in animals between 5- to 10-years-old (5/12 = 42%) and 1- to < 5-years-old (5/12 = 42%), followed by < 1-year-old (1/12 = 8%) and > 10 years-old (1/12 = 8%), whereas all cases in cats were observed from 5- to 10-years-old (2/2 = 100%).

Fifty percent (6/12) of the dogs studied had concomitant infection with canine morbillivirus and 33% (4/12) had no comorbidities, whereas for 17% (2/12) of dogs and all cats (2/2 = 100%), there was no information/missing data about the predisposing conditions/comorbidities (Table 2).

Cutaneous-subcutaneous lesions (8/12 = 67%), pneumonia (2/12 = 17%), and encephalitis (2/12 = 17%) were the clinical signs observed in dogs, whereas all cats presented skin lesions (2/2 = 100%), of which one case with concurrent osteomyelitis infection. Abscesses, fistulous tracts, cellulitis, and secretion of serous-to-purulent material containing white granules were the cutaneous lesions most observed in both dogs and cats. These clinical signs were mainly observed in the abdomen, dorsal, and ventral thoracic body regions. Hyperthermia, purulent nasal discharge, cough, and abnormal pulmonary sounds were

Table 1 Epidemiological data, clinical disorders, molecular speciation, and outcome of 14 nocardial infections in dogs and cats. Brazil, 2001–-2010

Animal			Epidemiological data			Clinical disorders	*Nocardia* species	Outcome
Companion species		Gender	Age (years-old)	Year	Predisposing factors/comorbidities			
Cats	1	Female	> 5- to < 10	2004	Unknown	Cellulitis, Osteomyelitis	*N. africana*	Died
	2	Male	> 5- to < 10	2008	Unknown	Cutaneous abcess	*N. veterana*	Unknown
Dogs	3	Female	> 5- to < 10	2000	None	Cutaneous abcess	*N. nova*	Died
	4	Male	> 5- to < 10	2001	Unknown	Cutaneous abcess	*N. farcinica*	Unknown
	5	Female	1- to < 5	2001	Morbillivirus[1]	Pneumonia	*N. nova*	Died
	6	Male	1- to < 5	2001	Morbillivirus[1]	Cutaneous abcess	*N. cyriacigeorgica*	Cure
	7	Female	1- to < 5	2001	Morbillivirus[1]	Cutaneous abcess	*N. nova*	Died
	8	Unknown	1- to < 5	2001	Morbillivirus[1]	Cutaneous abcess	*N. nova*	Died
	9	Male	> 5- to < 10	2004	None	Pneumonia	*N. veterana*	Unknown
	10	Male	> 5- to < 10	2008	None	Cutaneous abcess	*N. cyriacigeorgica*	Unknown
	11	Male	> 5- to < 10	2008	Unknown	Cutaneous abcess	*N. farcinica*	Unknown
	12	Unknown	1- to < 5	2009	Morbillivirus[1]	Cutaneous abcess	*N. cyriacigeorgica*	Died
	13	Unknown	> 10	2009	None	Encephalitis	*N. asteroides*	Died
	14	Unknown	< 1	2010	Morbillivirus[1]	Encephalitis, Pneumonia	*N. nova*	Died

Morbillivirus[1] = canine (distemper) morbillivirus infection

Table 2 In vitro antimicrobial susceptibility pattern of *Nocardia* species isolated from 14 diseased dogs and cats. Brazil, 2001–2010

Animal species Groups	Antimicrobials	Canine susceptibily profile			Feline susceptibility profile		
		S (%)	*I* (%)	*R* (%)	*S* (%)	*I* (%)	*R* (%)
Beta-lactams							
Cefalosporins	Ceftiofur	9/12 (75.3)	2/12 (16.4)	1/12 (8.3)	–	2/2 (100%)	–
	Cefuroxime	12/12 (100)	–	–	2/2 (100%)	–	–
	Ceftriaxone	9/12 (75.3)	1/12 (8.3)	2/12 (16.4)	–	2/2 (100%)	–
	Cephalexin	7/12 (58.3)	2/12 (16.4)	3/12 (25.3)	2/2 (100%)	–	–
Broad-spectrum penicillins	Amoxicillin/clavulanic acid	8/12 (66.4)	3/12 (25.3)	1/12 (8.3)	2/2 (100%)	–	–
	Ampicillin	4/12 (33.4)	1/12 (8.3)	7/12 (58.3)	–	2/2 (100%)	–
	Imipenem	10/12 (83.4)	1/12 (8.3)	1/12 (8.3)	2/2 (100%)	–	–
Aminoglycosides							
	Amikacin	10/12 (83.4)	1/12 (8.3)	1/12 (8.3)	–	2/2 (100%)	–
	Gentamycin	10/12 (83.4)	1/12 (8.3)	1/12 (8.3)	2/2 (100%)	–	–
Sulfonamides							
	Trimethoprim/sulfamethoxazole	9/12 (75.3)	1/12 (8.3)	2/12 (16.4)	–	2/2 (100%)	–

S, susceptible; *I*, intermediate; *R*, resistant; %, percentage

observed in dogs with bronchopneumonia, while seizures, nystagmus, hypermetria, incoordination, and broad-based gait were observed among dogs with encephalitis.

A high mortality rate (6/8 = 75%) was observed in canine cases with known outcome (Table 2). Three dogs (3/4 = 75%) with systemic signs of nocardiosis died, from which 66% (2/3) were also coinfected with morbillivirus. One cat (50%) with systemic nocardiosis also died, while another cat with a cutaneous lesion survived. Among all dogs diagnosed with morbillivirus, 83% (5/6) had a lethal outcome.

Cytological and histological examination

Cytological and histological examinations of different clinical specimens sampled revealed long or aggregate branching gram-positive, partially acid-fast, filamentous microorganisms, surrounded by an infiltrate of neutrophils, macrophages, lymphocytes, plasma and multinucleated cells, focal necrotic areas, and fibrous capsule, which characterize a pyogranulomatous inflammatory reaction.

Molecular diagnosis

PCR-based 16S rRNA gene resulted in the detection of *N. nova* (5/12 = 42%), *N. cyriacigeorgica* (3/12 = 25%), *N. farcinica* (2/12 = 17%), *N. veterana* (1/12 = 8%), and *N. asteroides* (1/12 = 8%) species in dogs, whereas *N. africana* and *N. veterana* were identified in cats (Table 2).

Discussion

We describe a case series study involving 12 dogs and two cats naturally infected by *Nocardia* species, emphasizing variable species detection, multidrug resistance pattern, and high mortality rates, particularly among dogs coinfected with morbillivirus and/or disseminated nocardial infections.

Cutaneous lesions were the major clinical disorder observed among the dogs and cats sampled, followed by pneumonia and encephalitis. These most common clinical signs may be the result of pathogen traumatic inoculation in cutaneous-subcutaneous tissues (throughout bite or scratch injuries), or inhalation of *Nocardia* from the environment. The wide distribution of the *Nocardia* species in the environment of the companion animals and the opportunistic nature of this bacteria [4, 27] reinforces the cutaneous lesions as one of the most important routes of transmission of the pathogen to dogs and cats [14, 17, 19].

Underlying conditions and coinfections with immunosuppressive or debilitating diseases are well-known predisposing factors for the development of clinical nocardiosis among companion animals [19], particularly dogs concomitantly infected by morbillivirus [15] and cats by feline leukemia virus (FeLV) or feline immunodeficiency virus (FIV) [9]. In this study, six dogs (6/12 = 50%) were coinfected with canine (distemper) morbillivirus, and no information/missing data on predisposing conditions or immunosuppressive infections in sampled cats were available. These results emphasize the poor prognosis of canine nocardiosis, especially among animals coinfected with canine morbillivirus that evolve to systemic dissemination of *Nocardia* [12, 13, 15].

Most dogs and cats sampled were male and grouped as young (1- to 5-years-old) or adult (5- to 10-years-old) animals. Likewise, in a study of nocardial infections in animals from the USA, 63.5% of dogs were young males [27], and a similar distribution was seen in a retrospective multi-institutional study of 17 cases of feline nocardiosis from Australia, in which 82.5% of diseased animals were males [9]. Indeed, canine and feline nocardiosis appears to be predominant in young-to-adult male animals, which may be related to higher environment exposure in reproductive periods or territorial aggressive behavior of cats, since males are probably more susceptible to traumatic inoculation of *Nocardia* species after bite or scratch injuries during fights [4, 17]

The routine diagnosis of canine and feline nocardiosis has been based on epidemiological data, clinical features, microbiological culture, imaging, and cytological/histological examinations [2, 4, 19]. Cytological and histological examinations of clinical specimens from companion animals sampled infected by *Nocardia* species revealed a pyogranulomatous (suppurative) inflammatory reaction, similar to previous reports in dogs [11–15] and cats [17, 19]. In fact, the virulence of *Nocardia* species that infect both domestic animals and humans has been attributed to a bacterial cell wall structure, the pathogen's ability to resist oxidative and enzymatic mechanisms inside phagocytic cells, and the development of suppurative and pyogranulomatous tissue reactions [3, 4, 7, 15, 18]. These different virulence mechanisms combined could justify the poor prognosis of nocardial infections in companion animals, particularly in those with systemic dissemination, a finding also observed in dogs sampled herein.

In regard to microbiological approaches, some phenotypic tests are time-consuming and not accurate appropriately to identify *Nocardia* at species-level [21]. Furthermore, advances in molecular-based diagnosis have enabled to distinguish among the clinically relevant species and the plethora of saprophytic *Nocardia* species [1, 5–7, 16, 18], including from companion animals [11–14, 17].

PCR-based 16S rRNA gene detected *N. nova* and *N. cyriacigeorgica* as the most frequent species in our 12 diseased dogs, followed by *N. farcinica*, *N. veterana*, and *N. asteroides* in minor occurrence, while isolated reports of *N. africana* and *N. veterana* were identified in two cats. The identification of *N. nova*, *N. veterana*, *N. cyriacigeorgica*, *N. farcinica*, and *N. africana* reinforce the pathogenicity of these species to companion animals [13, 14, 17]. Nonetheless, *N. otitidiscaviarum* [28], *N. brasiliensis* [29], and *N. pseudobrasiliensis* [12] reported in dogs and cats elsewhere were not identified among our animals. In this regard, 59% of cats had *N. nova* infection in a retrospective study of feline nocardiosis in Australia [9] although this species was not identified in our two cats. These discrepancies of species identification of *Nocardia* reported among companion animals from different countries may be credited to geographic variation of the pathogen [3, 9, 29], the accuracy of conventional phenotypic tests [30], or molecular methods to distinguish the different *Nocardia* at the species-level [11–14, 17]. Curiously, to our knowledge, molecular diagnosis of *N. veterana* in a cat sampled here is described for the first time, which may be related to the use of PCR-based 16S rRNA gene diagnosis; and highlight the importance of the introduction of molecular methods in routine diagnosis of nocardial infections from animal origin.

Nocardial infections in companion animals are usually refractory to conventional antimicrobial treatments because of the pathogen's intracellular location and its pyogranulomatous tissue reaction, which impairs therapeutic concentrations of drugs into lesion foci[4, 19]. Commonly, long-term therapy is required to nocardiosis in animals [9, 19] due to relapses after short treatment periods [4]. In addition, high lethality rates have been reported in canine and feline nocardiosis, probably related to concomitant infections with immunosuppressive viral pathogens that favor unresponsive therapy and poor prognosis [9, 12, 13, 15, 17, 30].

Aminoglycosides (amikacin, gentamicin), broad-spectrum beta-lactams, which included penicillins and derivatives (amoxicillin/clavulanic acid, imipenem), cephalosporins (ceftiofur, ceftriaxone, cefuroxime, cefotaxime), and potentiated sulfonamides (sulfamethoxazole/trimethoprim) appear to be first-choice classes/antimicrobials to treat nocardial infections in companion animals [19]. In fact, cefuroxime, imipenem, amikacin, gentamicin, and/or amoxicillin/clavulanic acid revealed high in vitro effectiveness against our *Nocardia* isolates recovered from diseased dogs and cats, and may be considered alternatives to treat nocardial infections in companion animals. Conversely, isolates showed high resistance to ampicillin and cephalexin, and multidrug resistance pattern was found in 36% isolates, reinforcing the importance to initiate therapy, if possible, based on previous in vitro susceptibility tests.

Nocardia-induced infections in humans are also seen as opportunistic, causing predominantly chronic bronchopulmonary disease [6]. *N. brasiliensis*, *N. asteroides*, *N. farcinica*, *N. otitisdiscaviarum*, *N. begingensis*, *N. nova*, and *N. abscessus* are species that have been described in human patients [20]. Nonetheless, besides an increased occurrence of human clinical infections, particularly in those patients with deficient cell-mediated immunity (e.g., people living with HIV/Aids, solid organ transplant recipients, diabetes, drug abuse, and malignancies) [7], nocardiosis has been recognized as a neglected disease [6], including in people without a history of predisposing risk factors [20]. Besides no clear evidence of pets-to-humans transmission of pathogenic *Nocardia*, the identification in the current study of *Nocardia* species that simultaneously affect humans and animals, in 14 diseased dogs and cats, represent relevance in human health due to close exposure of owners and their companion animals.

A convenience sample of companion animals infected by *Nocardia* species and no information/missing epidemiological data of some enrolled animals may be considered limitations of the present study.

Most descriptions of nocardial infections in dogs [4, 11–13, 15] and cats [16–18] are focused on case reports, and a restrict number of case series studies involving companion animals have been described worldwide [2, 9, 30]. In addition, nocardiosis may be considered an uncommon or rare disease in companion animals [4, 9]. Overall, we described a case series study involving 14 naturally *Nocardia*-acquired infections in dogs and cats, emphasizing variability of *Nocardia* species detected using a PCR-based 16S rRNA gene, occurrence of multidrug-resistant isolates, and a poor prognosis associated with a high mortality rate of canine nocardiosis, particularly among those systemically compromised and/or coinfected by canine morbillivirus.

Author contribution The authors contributed to the study review, data collection, and material preparation. The conception of the study was elaborated by Dr. Marcio Garcia Ribeiro. Material preparation and data collection were performed by Marcio Garcia Ribeiro, Agueda Castagna de Vargas, Marconi Rodrigues de Farias, Kung Dahr Chi, and Larissa Anuska Zeni Condas. Laboratorial molecular identification, antimicrobial pattern, histopathological, and description were analyzed by Katsukiyo Yazawa, Tetsuhiro Matsuzawa, Tohru Gonoi, Amanda Keller Siqueira, Tatiana Salerno, Juliana Werner, and Larissa Anuska Zeni Condas. All authors contributed to the written manuscript, with complementary review and editing of Guilherme Borges Bond. All authors read and approved the manuscript.

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Data Availability Complete epidemiological datas was presented in our tables, additional results of antimicrobial susceptibility results are available under request, and 16S rDNA sequences are available in NCBI public databank.

Declarations

Ethics approval This study was conducted under the Ethics Committee on Animal Use (CEUA) guidelines of the School of Veterinary Medicine and Animal Sciences, São Paulo University-UNESP, Botucatu, SP, Brazil (protocol number 169/2014).

Consent to participate/Consent for publication All authors of this paper are aware of the study developed, participated in thesis review, and contributed to the conclusions obtained in the study, further agreeing to its publication.

Competing interests The authors declare no competing interests.

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