

Species distribution and resistance profile of medical importance bacteria isolated from lesions of cats with sporotrichosis

Distribuição de espécies e perfil de resistência de bactérias de importância médica isoladas de lesões de felinos com esporotricose

Distribución de especies y perfil de resistencia de bacterias de importancia médica aisladas de lesiones felinas con esporotricosis

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Abstract

Sporotrichosis is an infection with zoonotic potential caused by the *Sporothrix schenckii* complex. It is widely distributed worldwide. The cutaneous form is the most common presentation of the disease. The cat is one of the main animals affected, having numerous fungal cells in its lesions, becoming an important fungi disseminator. Due to the type of skin lesion caused by the fungus, secondary bacterial infections commonly occur. The aim of the present study was to learn about the most common bacterial agents involved in feline lesions with sporotrichosis, as well as their resistance profile to antimicrobial agents. The faster the treatment of bacterial and fungal infections is controlled, the faster the lesions heal. Consequently, the transmission of the disease is controlled. Samples of skin lesions or nasal discharge from 18 domiciled or semi-domiciled cats diagnosed with sporotrichosis were collected. Cats were evaluated for secondary bacterial infections. The samples were processed to isolate aerobic bacteria that were later identified by MALDI TOF. Susceptibility was assessed using the disk diffusion method for a total of 17 antimicrobial agents. The main species identified were *Staphylococcus aureus* and *Staphylococcus felis*. *Proteus mirabilis* has also been identified in some samples. Among these microorganisms, two strains of *S. aureus* were identified as resistant to methicillin. The TSA's results showed sensitivity to most of the tested antimicrobials. *Staphylococcus* was the most identified genus in skin lesions and nasal secretions of cats with sporotrichosis. Most microorganisms were sensitive to fluoroquinolones and aminoglycosides. Penicillins and cephalosporins showed less potential for action on these bacteria, which showed greater resistance to these classes.

Keywords: Feline sporotrichosis; Bacteriology; MRSA; Zoonosis.

Resumo

A esporotricose é uma infecção com potencial zoonótico causada pelo complexo *Sporothrix schenckii*. É amplamente distribuído em todo o mundo. A forma cutânea é a apresentação mais comum da doença. O gato é um dos principais animais afetados, possuindo inúmeras células fúngicas em suas lesões, tornando-se um importante disseminador dos

fungos. Devido ao tipo de lesão cutânea causada pelo fungo, infecções bacterianas secundárias comumente ocorrem. O objetivo do presente estudo foi conhecer os agentes bacterianos mais comuns envolvidos em lesões de felinos com esporotricose, bem como seu perfil de resistência aos agentes antimicrobianos. Quanto mais rápido o tratamento de infecções bacterianas e fúngicas for controlado, mais rápido as lesões se consolidam. Consequentemente, a transmissão da doença é controlada. Foram coletadas amostras de lesões de pele ou secreção nasal de 18 gatos domiciliados ou semi-domiciliados com diagnóstico de esporotricose. Os gatos foram avaliados para infecções bacterianas secundárias. As amostras foram processadas para isolamento de bactérias aeróbias que foram posteriormente identificadas por MALDI TOF. A suscetibilidade foi avaliada pelo método de difusão em disco para um total de 17 agentes antimicrobianos. As principais espécies identificadas foram *Staphylococcus aureus* e *Staphylococcus felis*. *Proteus mirabilis* também foi identificado em algumas amostras. Dentre esses microrganismos, duas cepas de *S. aureus* foram identificadas como resistentes à meticilina. Os resultados dos TSA's mostraram sensibilidade à maioria dos antimicrobianos testados. *Staphylococcus* foi o gênero mais identificado nas lesões cutâneas e secreções nasais de gatos com esporotricose. A maioria dos microrganismos foi sensível as fluoroquinolonas e aminoglicosídeos. As penicilinas e cefalosporinas mostraram menor potencial de ação sobre essas bactérias, que apresentaram maior resistência a essas classes.

Palavras-chave: Esporotricose felina; Bacteriologia; MRSA; Zoonose.

Resumen

La esporotricosis es una infección con potencial zoonótico causada por el complejo *Sporothrix schenckii*. Se distribuye ampliamente en todo el mundo. La forma cutánea es la presentación más común de la enfermedad. El gato es uno de los principales animales afectados, presentando numerosas células fúngicas en sus lesiones, convirtiéndose en un importante diseminador de hongos. Debido al tipo de lesión cutánea causada por el hongo, comúnmente ocurren infecciones bacterianas secundarias. El objetivo del presente estudio fue conocer los agentes bacterianos más comunes implicados en las lesiones felinas con esporotricosis, así como su perfil de resistencia a los agentes antimicrobianos. Cuanto más rápido se controle el tratamiento de las infecciones bacterianas y fúngicas, más rápido se curarán las lesiones. En consecuencia, se controla la transmisión de la enfermedad. Se recolectaron muestras de lesiones cutáneas o secreciones nasales de 18 gatos domiciliados o semidomesticados diagnosticados de esporotricosis. Los gatos fueron evaluados para detectar infecciones bacterianas secundarias. Las muestras se procesaron para aislar bacterias aeróbicas que luego fueron identificadas por MALDI TOF. La susceptibilidad se evaluó mediante el método de difusión en disco para un total de 17 agentes antimicrobianos. Las principales especies identificadas fueron *Staphylococcus aureus* y *Staphylococcus felis*. También se ha identificado *Proteus mirabilis* en algunas muestras. Entre estos microorganismos, se identificaron dos cepas de *S. aureus* como resistentes a la meticilina. Los resultados de la TSA mostraron sensibilidad a la mayoría de los antimicrobianos probados. *Staphylococcus* fue el género más identificado en lesiones cutáneas y secreciones nasales de gatos con esporotricosis. La mayoría de los microorganismos fueron sensibles a las fluoroquinolonas y aminoglucósidos. Las penicilinas y cefalosporinas mostraron menor potencial de acción sobre estas bacterias, que mostraron mayor resistencia a estas clases.

Palabras clave: Esporotricosis felina; Bacteriología; MRSA; Zoonosis.

1. Introduction

Sporotrichosis is a zoonosis considered hyperendemic in the state of Rio de Janeiro/Brazil (Barros et al., 2008; Gremião et al., 2017; Schubach et al., 2004). Transmission is mainly through biting, scratching, and contact with the exudate present in lesions of infected cats (Barros et al., 2008; de Souza et al., 2018). The main agent of the disease are fungi from the *S. schenckii* complex (Barros et al., 2011). The main agent isolated from cat lesions in Brazil is *Sporothrix brasiliensis* (Boechat et al., 2018a; Oliveira et al., 2011; Rodrigues et al., 2013). Numerous fungal cells can be isolated from the surface of the ulcerated lesions of cats. This is important for the transmission by direct contact from one animal to another. The animal infected by the fungus can spread it in the environment through contact with plants or soil (da Cruz, 2013; Schubach et al., 2008).

In cats, the disease has different clinical forms. Skin lesions are the most common manifestation of this disease, with the appearance of nodules, gums, and ulcers (Scott et al., 1996). It is believed that lesions in the disease favor secondary infections by opportunistic bacteria (Houghton et al., 2005). According to Cavalcanti e Coutinho (2005), fungal skin diseases can predispose the animal to secondary bacterial infections. This secondary infection can worsen the lesion, increasing the time necessary for the closing and healing of lesions (Huang et al., 2014; Yeboah-Manu et al., 2013).

Staphylococcus spp. are resident members of the cutaneous microbiota and mucous membranes of men and animals. However, several species are also opportunistic pathogens that can cause serious skin diseases (Morris et al., 2008), and some studies have reported the possibility of exchanging genetic material between animal and human staphylococcal species capable of conferring antimicrobial resistance (Ferreira et al., 2011; Frank et al., 2009; Hemeg, 2021). No previous studies demonstrate the main bacteria related to secondary infection in sporotrichosis in cats or their relationship with the healing process. With this in mind, this study aimed to perform the identification and resistance profile of aerobic bacteria isolated from cutaneous lesions and nasal secretions of cats with sporotrichosis.

2. Methodology

Samples processing and bacterial identification:

Cats were selected (n = 18), regardless of gender, race, and age, who had ulcerated lesions and/or nasal discharge (rhinotracheitis), diagnosed with sporotrichosis. Animals were domiciled or stray cats of the city of Volta Redonda - RJ/Brazil. All of them had access to the street. The samples were collected during a period corresponding to July 2017 to March 2018.

All cats were diagnosed with Sporotrichosis by a cytological examination and culture. Samples selected presented concomitant neutrophilic inflammation or the presence of phagocytosis by neutrophils, macrophages, or eosinophils, which suggest the diagnosis of pyoderma. Samples were obtained from the cutaneous lesions or nasal secretion of the affected animals.

Samples of ulcerated lesions or nasal secretions were collected using a sterile swab with transport Stuart medium (ABSORVE, China). In animals that had multiple lesions, only one of the lesions was chosen to collect the material. Only one swab was collected from each animal. Swabs were transported to the Microbiology and Parasitology Laboratory at Severino Sombra University (USS) – Vassouras - Rio de Janeiro/Brazil, and processed within 24 hours.

Swabs were transferred to Blood Agar plates 5% of sheep's blood (Laborclin, Brazil) and incubated for 24 hours at 37 ° C. All colonies were visualized and evaluated for their morphotintorial characteristics. Those compatible with Gram-positive cocci were transferred to mannitol salt agar (Laborclin, Brazil). Colonies compatible with Gram-negative bacilli were transferred to EMB agar (Laborclin, Brazil). All isolates were then identified by *Matrix-Assisted Laser Desorption ionization-time of flight* (MALDI–TOF MS) following manufacturer orientation.

Antimicrobial susceptibility test (TSA):

All isolates were tested against different, non-hepatotoxic antibiotics, according to the recommendations of the Clinical and Laboratory Standards Institute - CLSI (CLSI, 2019; CLSI-VET, 2018). Antimicrobial profile of Gram-positive bacterias: oxacillin (1 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), levofloxacin (5 µg), ceftiofloxacin (30 µg), gentamicin (30 µg), penicillin G (10 U), amikacin (30 µg) and tobramycin (10 µg). To gram-negative bacterias were tested: ciprofloxacin (5 µg), ofloxacin (5 µg), levofloxacin (5 µg), tobramycin (10 µg), penicillin G (10 U), ampicillin (10 µg), ceftriaxone (30 µg), meropenem (10 µg), amoxicillin + clavulanic acid (20 µg), ampicillin + sulbactam (10 µg), cefazolin (30 µg) and ceftiofloxacin (30 µg).

3. Results

Identified microorganisms

- Isolates were obtained in 100% of the collected swabs (n = 18). Twenty different microorganisms were identified, mainly (90% - 18/20) as *Staphylococcus* genus. Among these, half (9/18) were identified as *S. felis*, 44.4% (eight) *S. aureus*, and 5.6% (one) *S. pseudintermedius*. Two Gram-negative isolates could be identified from two different animals as *Proteus mirabilis* (10% - 2/20). The results are summarized in Table 1.

Table 1: Relationship of bacteria isolated from feline lesions with sporotrichosis. The columns show the animal's identification, the isolation site and the identified microorganism.

Animal	Isolation site	Identified microorganism
Cat 1	Paw	<i>Staphylococcus felis</i>
Cat 2 ₁	Paw	<i>Proteus mirabilis</i>
Cat 2 ₂	Paw	<i>Staphylococcus aureus</i>
Cat 3	Paw	<i>Staphylococcus aureus</i>
Cat 4	Nasal discharge	<i>Staphylococcus aureus</i>
Cat 5	Head	<i>Staphylococcus felis</i>
Cat 6	Head	<i>Staphylococcus felis</i>
Cat 7	Nasal discharge	<i>Staphylococcus felis</i>
Cat 8	Nasal discharge	<i>Staphylococcus aureus</i>
Cat 9	Paw	<i>Staphylococcus felis</i>
Cat 10	Head	<i>Staphylococcus aureus</i>
Cat 11	Abdomen	<i>Staphylococcus felis</i>
Cat 12 ₁	Paw	<i>Proteus mirabilis</i>
Cat 12 ₂	Paw	<i>Staphylococcus aureus</i>
Cat 13	Paw	<i>Staphylococcus aureus</i>
Cat 14	Paw	<i>Staphylococcus felis</i>
Cat 15	Head	<i>Staphylococcus aureus</i>
Cat 16	Head	<i>Staphylococcus felis</i>
Cat 17	Upper body	<i>Staphylococcus felis</i>
Cat 18	Head	<i>Staphylococcus pseudintermedius</i>

* The subscribed numbers represent the animals which more than one bacterial strain was isolated from the samples collected. Source: Authors.

Susceptibility profile

- Of the 18 bacterial isolates of gram-positive cocci belonging to the genus *Staphylococcus* 100% (18/18) were sensitive to ciprofloxacin, ofloxacin, gentamicin, and tobramycin, 94.4% (17/18) were sensitive to levofloxacin (one isolate was considered susceptible, using increased exposure) and amikacin. Two *S. aureus* isolates showed resistance to methicillin, five to penicillin G, and one isolate of *S. felis* was resistant to penicillin G. Both *Proteus mirabilis* isolates demonstrated susceptibility to ampicillin, ceftriaxone, meropenem, amoxicillin + clavulanic acid, ampicillin + sulbactam, ofloxacin, tobramycin, ciprofloxacin, levofloxacin, and gentamicin. On the other hand, both isolates were resistant to ceftazolin and ceftiofur. The results of the susceptibility tests are compiled in Table 2 and Table 3, respectively.

Table 2: Profile of susceptibility to antimicrobials by bacterial isolate from *Staphylococcus* spp. of each animal, being sensitive (S), intermediate (I) and resistant (R).

Animal	Antimicrobial								
	OXA	CIP	OFX	LVX	CFO	GEN	AMI	TOB	PEN G
Cat 1	-	S	S	S	S	S	S	S	S
Cat 2 ₂	-	S	S	S	S	S	S	S	S
Cat 3	-	S	S	S	R	S	R	S	R
Cat 4	-	S	S	S	S	S	S	S	R
Cat 5	-	S	S	S	S	S	S	S	S
Cat 6	-	S	S	S	S	S	S	S	R
Cat 7	-	S	S	S	S	S	S	S	S
Cat 8	-	S	S	S	S	S	S	S	R
Cat 9	-	S	S	S	S	S	S	S	S
Cat 10	-	S	S	S	S	S	S	S	S
Cat 11	-	S	S	S	S	S	S	S	S
Cat 12 ₂	-	S	S	S	R	S	S	S	R
Cat 13	-	S	S	S	S	S	S	S	S
Cat 14	-	S	S	S	S	S	S	S	S
Cat 15	-	S	S	S	S	S	S	S	R
Cat 16	-	S	S	I	S	S	S	S	S
Cat 17	-	S	S	S	S	S	S	S	S
Cat 18	S	S	S	S	S	S	S	S	S

* Antimicrobial: oxacillin (OXA), ciprofloxacin (CIP), ofloxacin (OFX), levofloxacin (LVX), cefoxitin (CFO), gentamicin (GEN), amikacin (AMI), tobramycin (TOB) and penicillin G (PEN G). Source: Authors.

Table 3: Antimicrobial susceptibility profile by bacterial isolate of Gram-negative bacilli from each animal, being sensitive (S), intermediate (I) and resistant (R).

Animal	Antimicrobial											
	CVN	AMP	CFZ	CRO	MER	AMC	ASB	OFX	TOB	CIP	LVX	GEN
Cat 2 ₁	R	S	R	S	S	S	S	S	S	S	S	S
Cat 12 ₁	R	S	R	S	S	S	S	S	S	S	S	S

* Antimicrobial: ampicillin (AMP), ceftriaxone (CRO), meropenem (MER), amoxicillin + clavulanic acid (AMC), ampicillin + sulbactam (ASB), ofloxacin (OFX), tobramycin (TOB), ciprofloxacin (CIP), levofloxacin (LVX), gentamicin (GEN), cefazolin (CFZ) and cefovecin (CVN). Source: Authors.

4. Discussion

Zoonotic sporotrichosis caused by *S. brasilienses* is hyperendemic in Rio de Janeiro (Boechat et al., 2018b; Gremião et al., 2017). In cats, the most common form of sporotrichosis are multiple nodules and skin ulcers frequently associated with nasal mucosa lesions and respiratory signs. Also, low responses to treatment with itraconazole, the drug of choice for this disease, are commonly reported (Pereira et al., 2010). In this context, our study was the first to investigate bacteria present in

cat sporotrichosis. Also, the first to evaluate the antimicrobial susceptibility pattern of these bacteria. This can contribute to its treatment.

Studies on feline pyoderma are poorly reported in the literature compared to canine pyoderma. Few studies assess bacteria isolated from clinical lesions (Medleau & Blue, 1988; Scott et al., 2001). Pyoderma usually occurs secondary to other processes such as trauma, drugs, or illness that induces immunosuppression and a diversity of other dermatological problems (Mueller, 1999). Clinical lesions of pyodermas are quite variable and include pustules, furuncles, crusts, linear, and nodular ulcerative granulomatous lesions (Yu & Vogelnest, 2012). Therefore, may resemble the lesions caused by sporotrichosis. Due to lesions' characteristics, there is an imbalance in the cutaneous microbiota. We believe in the possibility of secondary bacterial infections in the lesions of cats affected by sporotrichosis.

Many of the animals from the present study had multiple bacterial cells in the cytological examination. Thus, it is assumed that the clinician should consider treatment with associated antibiotic therapy.

Most of the microorganisms isolated in this study belong to the genus *Staphylococcus* spp. A recent study showed that *Staphylococcus* is more isolated in sick cats than in healthy cats (Older et al., 2017). *S. felis* and *S. aureus* were the most prevalent species. Both species have already been isolated from the healthy skin and with skin lesions described in the literature (Baptiste et al., 2005; Devriese et al., 1984).

Although previous studies have indicated that *Staphylococcus* skin infections are less common in cats (Anette Loeffler et al., 2005; Rich, 2005), possibly due to decreased adherence to corneocytes (Lu & McEwan, 2007; Woolley et al., 2008), this study raises the possibility of a predisposition to *Staphylococcus* infection after skin trauma caused by sporotrichosis in cats.

Abraham et al. (2007) isolated 14 *S. aureus* (58% of 24 coagulase-positive *Staphylococcus* isolates) in 48 cats diagnosed with some inflammatory skin disease (ISD - Inflammatory Skin Disease), a smaller number of isolates of this species was obtained in healthy cats in the same study. This is in agreement with what our study found.

A greater amount of coagulase-negative *Staphylococcus* was isolated, a result also observed by Abraham et al. (2007) that isolated 98% of the coagulase-negative *Staphylococcus* from 48 cats diagnosed with ISD. Among coagulase-negative *Staphylococcus*, nine were *S. felis*, which have been reported causing otitis externa, cystitis, abscesses, wounds, and other skin infections (Igimi et al., 1989). Some studies have demonstrated the importance of infection by *S. felis* in cat lesions caused by flea-bite related hypersensitivity (Patel et al., 2002). *S. felis* may have played a role in skin disease since there was a systemic response to the antimicrobial therapy employed. Recently a study examined a single isolate of *S. felis* from a cat's subcutaneous wound and identified virulence factors such as biofilm and proteolytic enzymes (Kwaszewska et al., 2015). *S. felis* was the most isolated species in healthy cats and cats classified as having some disease in a study by Bierowiec et al. (2019).

Proteus mirabilis was the only gram-negative bacilli identified. Infections by this genus are sometimes neglected (O'Hara et al., 2000). Due to fecal elimination and free access to the street and, consequently, contact with the soil and unhealthy environments, these microorganisms assume typically opportunistic behavior, as described in animals and humans (O'Hara et al., 2000). Devitalization of tissues and injuries are considered predisposing factors for the occurrence of infections by *P. mirabilis* (Quinn et al., 2005), making the animal a carrier of lesions caused by sporotrichosis predisposed to these infections.

The highest resistance level was observed for Penicillin G, with 33.3% of the 18 isolates of *Staphylococcus* spp. This is the expected result since the production of β -lactamases by this genus is frequent. Similar rates were observed elsewhere (Qekwana et al., 2017). In recent years, several studies have demonstrated the acquisition of multidrug resistance by *Staphylococcus* spp (A Loeffler & Lloyd, 2010; Springer et al., 2009). even methicillin-resistant *S. aureus* (MRSA) in

companion animal and your zoonotic potential (A Loeffler & Lloyd, 2010; Qekwana et al., 2017). Two strains of *S. aureus* showed resistance to cefoxitin in this study. The TSA result shows a high degree of sensitivity to fluoroquinolones and aminoglycosides. However, it is necessary to monitor the isolation profiles and susceptibility to antimicrobials in veterinary practice, to optimize the cure rate and the rational use of antibiotics.

The number of samples collected and the lack of minimum inhibitory concentration for the tested antimicrobials were some limitations of the present study. The *mecA* gene for confirmation of bacteria methicillin could also be very clarifying. Further studies with more cats may clarify the role of bacteria in the feline sporotrichosis.

5. Conclusion

Staphylococcus spp. was the most identified genus in skin lesions and nasal secretions of cats with sporotrichosis, with *S. aureus* and *S. felis* being the most prevalent species. Fluoroquinolones and aminoglycosides were more sensitive, with penicillins and cephalosporins being the antimicrobials that showed greater resistance. It is essential to carry out the resistance profile of the bacteria identified. This should be mandatory for responsible antibiotic therapy. Studies that identify virulence and phenotypic and genotypic resistance factors of bacteria associated with lesions of cats with sporotrichosis are necessary to understand the interaction of bacteria with fungi of the *S. schenckii* complex.

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