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Short communication

The oral microbiota of domestic cats harbors a wide variety of *Staphylococcus* species with zoonotic potential

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ABSTRACT

This study aimed to characterize the species, antimicrobial resistance and dispersion of CRISPR systems in staphylococci isolated from the oropharynx of domestic cats in Brazil. *Staphylococcus* strains (n = 75) were identified by MALDI-TOF and sequencing of *rpoB* and *tuf* genes. Antimicrobial susceptibility was assessed by disk diffusion method and PCR to investigate the presence of antimicrobial-resistance genes usually present in mobile genetic elements (plasmids), in addition to plasmid extraction. CRISPR – genetic arrangements that give the bacteria the ability to resist the entry of exogenous DNA – were investigated by the presence of the essential protein Cas1 gene. A great diversity of *Staphylococcus* species (n = 13) was identified. The presence of understudied species, like *S. nepalensis* and *S. pettenkoferi* reveals that more than one identification method may be necessary to achieve conclusive results. At least 56% of the strains contain plamids, being 99% resistant to at least one of the eight tested antimicrobials and 12% multidrug resistant. CRISPR were rare among the studied strains, consistent with their putative role as gene reservoirs. Moreover, herein we describe for the first time their existence in *Staphylococcus lentus*, to which the system must confer additional adaptive advantage. Prevalence of resistance among staphylococci against antimicrobials used in veterinary and human clinical practice and the zoonotic risk highlight the need of better antimicrobial management practices, as staphylococci may transfer resistance genes among themselves, including to virulent species, like *S. aureus*.

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1. Introduction

Cats are among the most common domestic animals in the world, totaling more than 500 million individuals living with humans, in a relationship dated from thousand years ago, as evidenced by fossil remains (Vigne et al., 2016). Pets can carry a variety of bacteria, worms, viruses and fungi. In addition, cats' outdoor habits contribute for them to carry greater numbers and more diverse microbiota than dogs (Buma et al., 2006). Because of the intimate relationship that cats and humans may have, many of these microrganisms can be transmitted from pet to owner and vice-versa, including important pathogens like methicillin-resistant *Staphylococcus aureus* (MRSA), responsible for several hard-to-treat infections in humans (Muniz et al., 2013).

Most of the almost 50 species of the *Staphylococcus* genus are harmless residents of the normal microbiota of skin and mucous membranes of mammals (Becker et al., 2014). However, species sharing the same environment may exchange genetic material among themselves, especially plasmids, providing genes that confer antimicrobial resistance to pathogenic species, such as *S. aureus* (Rossi et al., 2016). Thus, species historically and mistakenly referred to as inoffensive can threat human health for acting as reservoirs of genes for closely related pathogens (Otto, 2013). We hypothesize that this characteristic must be accompanied by the absence of CRISPR systems, which are arrays of repeated sequences and associated genes that give the bacterium the ability to develop adaptive immunity against exogenous genetic material, like plasmids and bacteriophages (Marraffini, 2015).

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Understanding the bacterial populations that inhabit companion animals is an important way of restraing their spreading and securing the health of both pet and human. Thus, the aims of this work were i) to identify a population of *Staphylococcus* spp. isolated from different cats in Rio de Janeiro (Brazil) and ii) to characterize their antimicrobial resistance and CRISPR content in order to evaluate the potential threat that these microorganisms may signify to the cats and their owners.

2. Materials and methods

2.1. Microorganisms, culture conditions and DNA purification

We analyzed 75 strains belonging to the Culture Collection of Bacteria of Veterinary Interest of the Laboratory of Veterinary Bacteriology of Universidade Federal Fluminense (www.labv.uff.br). Those were previously collected from the oropharynx of different clinically healthy adult cats from Rio de Janeiro and characterized by biochemical methods as belonging to the genus *Staphylococcus* (Muniz et al., 2013). All strains were cultivated at 37 °C for 24 h in brain-hearth infusion (BHI) before the tests. Genomic DNA was isolated with the Wizard™ Genomic DNA Purification Kit (Promega, USA) following the manufacturer's instructions.

2.2. Bacterial species identification

The identification of the bacterial strains was performed by combining different molecular approaches. First, the differentiation of protein profiles of bacterial cultures was analyzed in triplicate by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry (MALDI-TOF) as previously described (Tomazi et al., 2014). Mass spectral data were collected within the *m*/*z* range of 2000–20,000, and the data were acquired using the FlexControl software 3.3 (Bruker Daltonics). MALDI-TOF results were complemented and confirmed by the partial sequencing of the *rpoB* and *tuf* genes. Briefly, these target genes were amplified from 25 ng of DNA by PCR with the primer pairs rpoBF/rpoBR (Drancourt and Raoult, 2002) and tufF/tufR (Heikens et al., 2005) and the GoTaq® G2 Green Master Mix (Promega, USA), following the manufacturer's protocols. Amplification steps for the *rpoB* gene were performed as indicated by the primers authors specified above.

2.3. Antimicrobial susceptibility testing and plasmid extraction

The susceptibility of the strains was tested by the disk diffusion method against eight antimicrobials of different classes: aminoglycosides (gentamicin), β-lactams (oxacillin and ampicillin), amphenicols (chloramphenicol), cephalosporins (cefoxitin), tetracyclines (tetracycline), macrolides (erythromycin) and mupirocin, (Cefar, Brazil). All tests were performed according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2015). Because of the lack of breakpoints for mupirocin in CLSI, susceptibility and differentiation of levels of resistance to this antibiotic were interpreted following the criteria previously established by our group (Oliveira et al., 2007). In order to correlate the resistance profiles with the presence of mobile genetic DNA, all strains were submitted to plasmid extraction, following protocols established by our group (Giambiagi-Marval et al., 1990).

2.4. PCR for antimicrobial resistance genes

The presence of resistance genes commonly encountered in mobile genetic elements was investigated by PCR. All reactions were performed with the GoTaq® G2 Green Master Mix (Promega, USA), 25 ng of purified DNA and primers specified below. The *S. haemolyticus* strain MD2 (Rossi et al., 2016) was used as positive control for all reactions. The aminoglycoside-resistance genes *aadD* and *aacA-aphD*, respectively coding for the aminoglycoside adenyltransferase AadD and the bifunctional aminoglycoside modifying enzyme AacA-AphD, were amplified with the primer pairs aadDF/aadDR and aac6-aph2aFw/aac6-aph2aRev (Schiwon et al., 2013), following the amplification steps suggested by the author. The methicillin-resistance gene *mecA* was amplified with the primer pair MRS1/MRS2, as recommended by the author (Del Vecchio et al., 1995). Finally, the mupirocin-resistance gene *mupA* was amplified with the primer pair M1/M2, following the amplification steps preconized by Nunes et al. (1999).

2.5. Investigation of CRISPRs

The dispersion of CRISPR systems among the 75 strains was examined by PCR for the detection of the essential CRISPR-associated protein Cas1. A cas1F (5′-AGAAGCACAGGCTGCAAGAA-3′) and cas1R (5′-TCACACTATCAAGTAACCTCACCA-3′) primer pair was designed based on a conserved region of the gene obtained from the alignment made with the sequences of the following

Table 1
Species identification of *Staphylococcus* strains used in this work by MALDI-TOF complemented with sequencing of the *tuf* and *rpoB* genes.

MALDI-TOF (number of strains)	Definitive identification (*tuf* and/or *rpoB* genes)[a] (number of strains)	Confidence of MALDI-TOF results
S. cohnni (5)	*S. cohnni* (5)	100%
S. haemolyticus (1)	*S. haemolyticus* (1)	100%
S. pettenkoferi (1)	*S. pettenkoferi* (1)	100%
S. saprophyticus (1)	*S. saprophyticus* (1)	100%
S. sciuri (5)	*S. sciuri* (5)	100%
S. xylosus (2)	*S. xylosus* (2)	100%
S. felis (1)	*S. felis* (1)	100%
S. lentus (18)	*S. lentus* (16), *S. nepalensis* (1), *S.* spp. (1)	88,9%
S. nepalensis (6)	*S. nepalensis* (5), *S.* spp. (1)	83,3%
S. epidermidis (4)	*S. epidermidis* (3), *S.* spp. (1)	75%
S. aureus (3)	*S. aureus* (2), *S. lentus* (1)	66,7%
S. equorum (6)	*S. equorum* (4), *S. nepalensis* (1), *S. lentus* (1)	66,7%
S. fleurettii (2)	*S. fleurettii* (1), *S.* spp. (1)	50%
S. lugdunensis (1)	*S. lentus* (1)	0%
S. arlettae (1)	*S. nepalensis* (1)	0%
Not determined[b] (18)	*S. nepalensis* (7), *S. lentus* (3), *S. xylosus* (2), *S. cohnni* (1), *S. sciuri* (1), *S.* sp. (4)	not applicable

[a] Species were defined when at least two of the three identification techniques were concordant. Strains with inconclusive results to the species level were designated as *S.* spp.
[b] Results yielded from MALDI-TOF were unreliable (scores ≤ 1.699).

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