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Antimicrobial susceptibility profiles of *Staphylococcus* spp. from domestic and wild animals

Perfil de suscetibilidade antimicrobiana e diversidade de *Staphylococcus* spp. de animais domésticos e silvestres

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— NOTE —

ABSTRACT

*The aim of this study was to determine the prevalence and diversity of veterinary clinical isolates of **Staphylococcus** and analyze their antimicrobial susceptibility. One hundred **Staphylococcus** spp. clinical isolates from domestic and wild animals were subjected to partial sequencing of the 16S rRNA gene to species determination. Antimicrobial susceptibility was obtained by a disk diffusion test against six antibiotics: amoxicillin (AMX), cephalexin (LEX), ciprofloxacin (CIP), erythromycin (ERY), gentamicin (GEN) and trimethoprim-sulfamethoxazole (SXT). The most common specie was **S. pseudintermedius** (61%, 61/100) and resistance to ERY (57%, 57/100), SXT (50%, 50/100) and AMX (46%, 46/100) was detected most frequently. In total, 40% (40/100) of **Staphylococcus** spp. exhibited a multidrug-resistant (MDR) phenotype. Results of this study emphasize that animals are reservoir of MDR **Staphylococcus** spp.*

Key words: ***Staphylococcus** spp., antimicrobial resistance, multidrug resistance.*

RESUMO

*O objetivo do presente trabalho foi determinar a prevalência e diversidade de isolados clínicos veterinários de **Staphylococcus** e analisar o perfil de suscetibilidade a antimicrobianos. Um total de 100 **Staphylococcus** spp. isolados de amostras clínicas de animais domésticos e silvestres foram submetidos ao sequenciamento parcial do gene 16S rRNA, para determinação da espécie. A suscetibilidade antimicrobiana foi obtida por meio da técnica de Disco Difusão contra seis antibióticos: amoxicilina, cefalexina, ciprofloxacina, eritromicina, gentamicina e sulfazotrim. A espécie mais frequente foi **S. pseudintermedius** (61%, 61/100) e a resistência à eritromicina (57%, 57/100), Sulfazotrim (50%, 50/100) e Amoxicilina (46%, 46/100) foi detectada mais frequentemente. No total, 40% (40/100) dos **Staphylococcus** spp. demonstraram um fenótipo de multirresistência a drogas (MRD). Os resultados obtidos neste trabalho reforçam o fato de que animais são reservatórios de **Staphylococcus** spp. MRD.*

Palavras-chave: ***Staphylococcus** spp., resistência a antimicrobianos, multirresistência a drogas.*

Multidrug-resistant ***Staphylococcus*** spp. isolates from humans and animals have been reported in recent decades, and these pose a challenge not only in human medicine but also in veterinary medicine. GÓMEZ-SANZ et al. (2013) showed the presence of traditionally human strains of ***S. aureus*** in dogs, which reflect the capacity of these strains to adapt to different hosts. The increasing evidence of ***S. aureus*** and ***S. pseudintermedius*** that are resistant to many drugs, particularly oxacillin, is a serious problem in treatment and control of staphylococcal infections (DURAN et al., 2012).

Studies of the antimicrobial resistance profiles of ***Staphylococcus*** spp. are very useful and can provide data that assist with strategies for avoiding the dispersion of these multidrug-resistant (MDR) microorganisms. In this context, the aims of this study were (1) to determine the prevalence and diversity of clinical isolates of ***Staphylococcus*** from domestic and wild animals and (2) to analyze their antimicrobial susceptibility.

The study was performed in the Veterinary Microbiology Laboratory at Universidade Federal de Mato Grosso (UFMT), between 2012 and 2013. One hundred isolates of ***Staphylococcus*** were obtained

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from swabs and biopsies of lesions at different sites (abscess, cornea, ear, fluids, fracture, lung, lymph node, oral mucosa, nail, rectum, skin, and urine) of domestic and wild animals (Cockatiel, Crab-eating fox, Crab-eating raccoon, Hoary fox, Maned wolf and Rabbit). Samples were inoculated into eight per cent sheep blood agar plates (Sigma-Aldrich) according QUINN et al. (1994), and incubated at 37°C for 48h. Identification of staphylococci was on the basis of colony morphology, Gram staining and biochemical tests such catalase test (QUINN et al., 1994). DNA extraction was performed using the phenol-chloroform extraction method (SAMBROOK & RUSSELL, 2001).

Species were identified using 16S rRNA partial sequence analysis, according to LANE (1991). Sequences obtained by ABI 3500 Genetic Analyzer (Applied Biosystems) were compared to the sequences available in GenBank using BLAST <http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>. Positive control was an American Type Culture Collection (ATCC) ***S. aureus*** strain 25923 and ultrapure water was negative control.

Antimicrobial susceptibility tests were performed using disk diffusion on Mueller-Hinton agar. All procedures and interpretation of antibiotic susceptibility were followed as previously described by the Clinical and Laboratory Standards Institute (CLSI) documents M100-S22 (CLSI, 2012) and VET01-A4 (CLSI, 2013). The following antibiotic disks (Cefar Diagnóstica Ltda) were tested: gentamicin (10µg, GEN), cephalexin (30µg, LEX), erythromycin (15µg, ERY), ciprofloxacin (5µg, CIP), amoxicillin (10µg, AMX) and trimethoprim-sulfamethoxazole (25µg, SXT). Isolates resistant to three or more antimicrobial classes were classified as MDR (MAGIORAKOS et al., 2012).

Association between phenotypic resistance, host characteristics (species and site of infection) and ***Staphylococcus*** species were evaluated by the $\varkappa^2$ test and Fisher's exact test, using the software R (R-3.0.2). Differences were considered significant when $P<0.05$.

Coagulase positive ***Staphylococcus*** species such as ***S. pseudintermedius*** (61%, 61/100), ***S. schleiferi*** (15%, 15/100), ***S. aureus*** (5%, 5/100), and ***S. delphini*** (3%, 3/100) were more commonly detected than coagulase negative species (Table 1). Similar results were reported by DETWILER et al. (2013).

Isolates were more frequently associated to canine samples (78%, 78/100) followed by wild animals (9%, 9/100) and cats (8%, 8/100). ***S. pseudintermedius*** (68%, 53/78) and ***S. schleiferi*** (18%, 14/78) were associated mainly with canine samples. Other species like ***S. cohnii***, ***S. delphini***, ***S. arlettae*** and ***S. pasteuri*** that occasionally or rarely were associated to disease in animals were detected from lesions.

S. sciuri (3%, 3/100) were isolated only in wild animals (***Cerdocyon thous***, ***Lycalopex vetulus***, ***Chrysocyon brachyurus***). This species was associated to mastitis in dairy cattle (RAHMAN et al., 2005) and have been reported in wild animals, such as rodents and insectivores (HAUSCHILD & SCHWARZ, 2003), but not in wild canidae.

Drug resistance frequencies were described in figure 1. ERY (57%, 57/100), SXT (50%, 50/100) and AMX (46%, 46/100) were more frequent resistant and LEX (12%, 12/100) was less frequent. In a study performed in Tunisia using ***S. pseudintermedius*** isolates from healthy dogs, only 1.8% of isolates were erythromycin-resistant (GHARSA et al., 2013); however, DÉGI et al. (2013) studied otitis isolates from dogs in Romania and reported that 61.3% of isolates were resistant to this drug and YOON et al. (2010) in Korea observed 60.8% of resistance.

Forty isolates exhibited an MDR phenotype (Table 1) and the most common associations were AMX-ERY-SXT (17,5%, 7/40), AMX-CIP-ERY-GEN-SXT (17,5%, 7/40) and AMX-CIP-ERY-SXT (15%, 6/40). This result was higher than the result reported by GHARSA et al. (2013) (18%) but lower than the result reported by BARDIAU et al. (2013) in isolates from animals in Japan (100%) and by YOUN et al. (2011) in Korea (71.9%). In a study of 103 MRSP (methicillin-resistant ***Staphylococcus pseudintermedius***) isolates from dogs from several countries in Europe, USA and Canada, resistance to multiple antimicrobials routinely used in pets was observed (PERRETEN et al., 2010).

Emergence of ***Staphylococcus*** spp. multidrug resistance, especially oxacillin-resistant, can complicate treatment of infections in animals such as otitis, dermatitis, urinary tract infections, leading to recurrent disease. Furthermore, the transmissions of these strains from animals to humans and from humans to animals are potential risks that have to be considered. Transmission of ***S. pseudintermedius*** between dogs and their owners was recently reported (GÓMEZ-SANZ et al., 2011). No significant association was observed ($P>0.05$) between ***Staphylococcus*** species, host type and resistance profile.

Identification of staphylococci species by conventional methods requires a minimum of two days period or more, can be influenced by

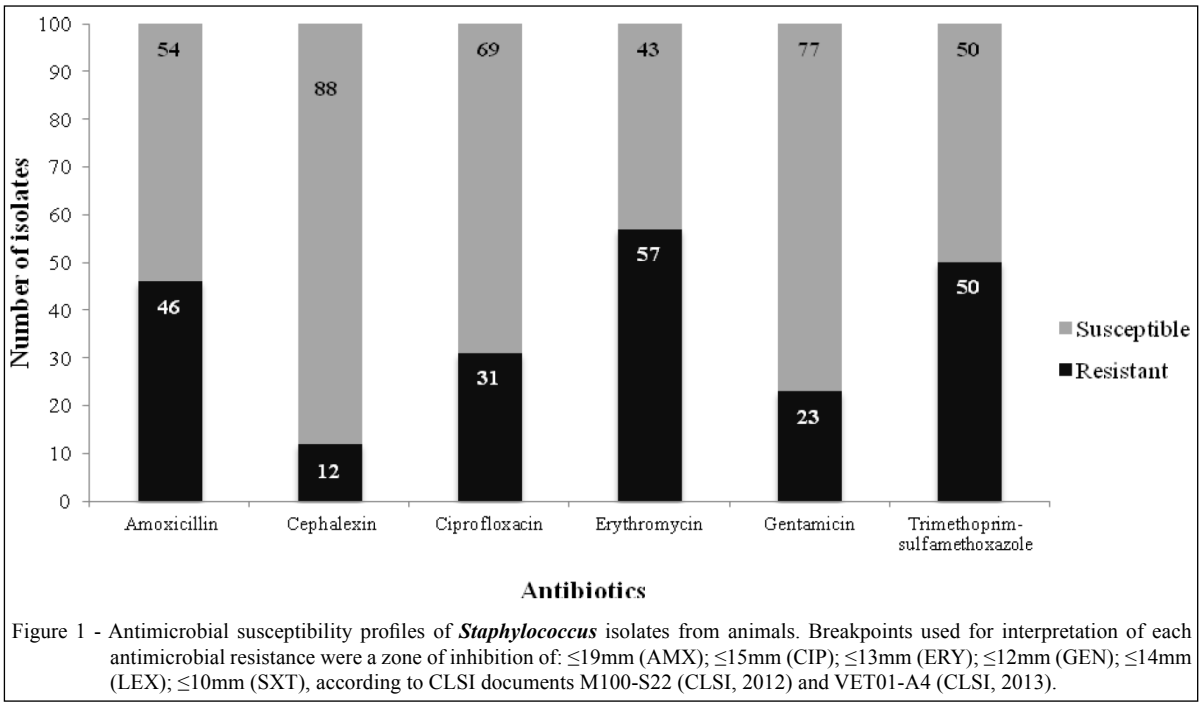

Figure 1 - Antimicrobial susceptibility profiles of ***Staphylococcus*** isolates from animals. Breakpoints used for interpretation of each antimicrobial resistance were a zone of inhibition of: ≤19mm (AMX); ≤15mm (CIP); ≤13mm (ERY); ≤12mm (GEN); ≤14mm (LEX); ≤10mm (SXT), according to CLSI documents M100-S22 (CLSI, 2012) and VET01-A4 (CLSI, 2013).

different variables and different species with similar phenotypic features. For these reasons, molecular methods, like partial sequence analysis of 16S rRNA gene, have been used to confirm the results obtained in the phenotypic tests (FORSMAN et al., 1997). Regarding the antimicrobial susceptibility tests, the disk diffusion is a simple and practical method used in many veterinary diagnostic laboratories; however, the results obtained are only qualitative. Broth microdilution is a standard reference method (Minimal Inhibitory Concentration-MIC) that can be used to confirm and measure quantitatively the *in vitro* activity of an antimicrobial agent against bacterium isolates (CLSI, 2013, VET01-A4).

In conclusion, this study emphasize that animals are reservoir of MDR ***Staphylococcus*** spp. and may become an important source of contamination of these microorganisms to both humans and other animals.

Table 1 - Prevalence and multidrug-resistant phenotype of ***Staphylococcus*** species from animal samples.

	------Bovine------		------Canine------		------Equine------		-------Feline-------		------Swine-------		--Wild animals--	
	n	MDR	n	MDR	n	MDR	n	MDR	n	MDR	n	MDR
S. arlettae	0	0	1	1	0	0	0	0	0	0	0	0
S. aureus	1	0	1	0	1	0	1	1	0	0	1[a]	0
S. cohnii	0	0	1	0	0	0	0	0	0	0	0	0
S. delphini	0	0	2	1	0	0	0	0	0	0	1[b]	1[b]
S. epidermidis	1	1	1	1	0	0	0	0	0	0	2[b,c]	1[c]
S. felis	0	0	3	1	0	0	1	0	0	0	0	0
S. pasteuri	0	0	1	0	0	0	0	0	0	0	0	0
S. pseudintermedius	1	0	53	23	0	0	4	4	1	1	2[c,d]	0
S. schleiferi	0	0	14	3	0	0	1	1	0	0	0	0
S. sciuri	0	0	0	0	0	0	0	0	0	0	3[c,e,f]	0
S. simulans	0	0	1	0	0	0	1	0	0	0	0	0

[a]***Oryctolagus cuniculus*** (Rabbit); [b]***Nymphicus hollandicus*** (Cockatiel); [c]***Cerdocyon thous*** (Crab-eating fox); [d]***Procyon cancrivorus*** (Crab-eating raccoon); [e]***Lycalopex vetulus*** (Hoary fox); [f]***Chrysocyon brachyurus*** (Maned wolf). MDR- Multidrug-resistant. n - Number of samples.

Thus, monitoring and surveillance programs related to dispersion of these microorganisms are needed.

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