Faculdade de Medicina de São José do Rio Preto
Programa de Pós-Graduação em Ciências da Saúde

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Ocorrência de bactérias *Staphylococcus* spp. Meticilina-Resistentes (MRS) em Animais da Região de São José do Rio Preto, SP.

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SUMÁRIO

Ao meu marido, por ser meu alicerce, meu

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Meu amor por você é eterno.

Aos meus filhos Vicente e Vitório,

vocês são responsáveis por toda vida e amor que pulsam em mim.

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"Tudo tem o seu tempo determinado, e há tempo para todo o propósito debaixo do céu".

Eclesiastes 3:1

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Lista de Abreviaturas e Símbolos

%	Porcentagem
<	Menor que
≤	Menor ou igual
ºC	Graus Celsius
®	Sinal registrado
β	Beta
µg	Micrograma
µL	Microlitro
ANVISA	Agência Nacional de Vigilância Sanitária
UFC	Unidade formadora de colônias
CIAPAV	Centro de Análises e Patologia Veterinária
CoNS	*Staphylococcus* coagulase-negativo
CoPS	*Staphylococcus* coagulase-positivo
A.F	Absolute frequency
R.F	Relative frequency
h	Hora
mm	Milímetro
MRCoNS	*Staphylococcus* meticilina-resistentes coagulase-negativos
MRCoPS	*Staphylococcus* meticilina-resistentes coagulase-positivos
MRS	*Staphylococcus* meticilina-resistentes
MRSA	*Staphylococcus aureus* meticilina-resistente

n	Número
N.I.	Not informed
OMS	Organização Mundial da Saúde
PAN-BR	Plano de Ação Nacional para Prevenção e Controle da Resistência aos Antimicrobianos do Brasil
PBP2a	Proteína ligadoras de penicilina adicional
PBPs	Proteína ligadoras de penicilina
RAM	Resistência a antimicrobianos
S	Sensível
SCCmec	*Staphylococcal cassette chromossome mec*
spp.	Espécies
UFC/ml	Unidade formadora de colônia por mililitro
WHO	*World Health Organization*

Resumo

Introdução: A resistência bacteriana é agravada pelo uso inadequado de antibióticos que amplia a propagação de genes de resistência para além dos microrganismos causadores de doenças, o que acaba limitando severamente as opções de tratamento disponíveis, expondo a saúde pública a riscos. Os *Staphylococcus* apresentam ampla distribuição e estão adaptados a sobreviver tanto no ambiente quanto no microbioma de seres humanos e animais. Essas bactérias são responsáveis por uma variedade de infecções em animais. A manifestação dos *Staphylococcus* varia conforme diversos fatores de interação, incluindo a imunidade do hospedeiro e a capacidade de resistência a antibióticos. **Objetivos:** Investigar a ocorrência de *Staphylococcus* meticilina-resistente (MRS) em animais atendidos em clínicas veterinárias; identificar dentre os *Staphylococcus*, os grupos mais comuns nas infecções animais e examinar as características fenotípicas de resistência dos isolados aos antimicrobianos avaliados em teste de antibiograma. **Material e Métodos:** A pesquisa usou dados secundários de laudos de culturas e antibiogramas de amostras biológicas de animais atendidos em clínicas veterinárias de São José do Rio Preto, SP e região. Foi uma pesquisa documental retrospectiva, com abordagem principalmente quantitativa. A análise estatística descritiva foi realizada a partir dos cálculos das medidas de tendência central, dispersão e contagens de frequências. **Resultados:** Foram considerados apenas os laudos que indicaram presença de MRS, totalizando 118 casos, dos quais 94 eram cães (79,7%), 18 gatos (15,3%), uma ave (0,8%), dois bovinos (1,7%), dois equinos (1,7%) e um lagomorfo (0,8%). Dos isolados totais de MRS analisados, foi observado que 73 (61,9%) eram coagulase-negativos, seguidos por 25 (21,2%) de *S.aureus* e 20 (16,9%) de outras espécies de coagulase-positivos. As maiores observações foram em conduto auditivo, representando

28,8% das amostras biológicas. **Conclusões:** Este é o primeiro estudo de MRS em animais da região de São José do Rio Preto, SP. As bactérias examinadas no estudo apresentaram resistência a β-lactâmicos, assim como, a outras classes de medicamentos como macrolídeos, clindamicina e quinolonas. A monitoração constante da resistência bacteriana é crucial para entender padrões de resistência e prevenir riscos de saúde pública.

Palavras-Chave: *Staphylococcus* Meticilina-resistentes; *Staphylococcus* Coagulase-negativos; Animais Domésticos; β-lactâmicos.

Abstract

Introduction: Bacterial resistance is aggravated by inappropriate use of antibiotics, which amplifies the spread of resistance genes beyond disease-causing microorganisms, which ends up severely limiting the available treatment options, exposing public health risks. *Staphylococci* are widely distributed and are adapted to survive both in the environment and in the microbiome of humans and animals. These bacteria are responsible for a variety of infections in animals. The manifestation of *Staphylococcus* varies according to several interacting factors, including host immunity and resistance to antibiotics. **Objectives:** To investigate the occurrence of methicillin-resistant *Staphylococcus* (MRS) in animals treated at veterinary clinics, to identify among the Staphylococcus the most common groups in animal infections and to examine the phenotypic characteristics of resistance of the isolates to the antimicrobials evaluated in the antibiogram test. **Material and Methods:** The research used secondary data from culture reports and antibiograms of biological samples from animals treated at veterinary clinics in São José do Rio Preto, SP and region. It was a retrospective documentary research, with a mainly quantitative approach. Descriptive statistical analysis was performed based on calculations of measures of central tendency, dispersion and frequency counts. **Results:** Only the reports that indicated the presence of MRS were considered, totaling 118 cases, of which 94 were dogs (79.7%), 18 cats (15.3%), one bird (0.8%), two cattle (1. 7%), two horses (1.7%) and one lagomorph (0.8%). Of the total MRS isolates analyzed, it was observed that 73 (61.9%) were coagulase-negative, followed by 25 (21.2%) of *S.aureus* and 20 (16.9%) of other coagulase-positive species. The largest observations were in the auditory canal, representing 28.8% of the biological samples. **Conclusions.** This is the first study of MRS in animals in the region of São José

do Rio Preto, SP. The bacteria examined in the study exhibited resistance to β-lactams, as well as other drug classes such as macrolides, clindamycin and quinolones. Constant monitoring of bacterial resistance is crucial to understanding resistance patterns and preventing public health risks.

Keywords: Methicillin-resistant *Staphylococcus*; Coagulase-negative *Staphylococcus*; Domestic animals; β-lactams.

1.INTRODUÇÃO

1. INTRODUÇÃO

1.1 Resistência Antimicrobiana

O aumento e a disseminação de microrganismos resistentes a antimicrobianos representam uma preocupação mundial, tanto na medicina humana quanto na veterinária. No Brasil, apesar da grande preocupação com a resistência a antimicrobianos (RAM) nos animais de companhia e de produção de alimentos, bem como, suas implicações em saúde pública, ainda há necessidade de informações epidemiológicas sobre os microrganismos que transitam e atuam fora do ambiente hospitalar (1,2).

A RAM acarreta impacto financeiro significativo nas economias nacionais e nos sistemas de saúde, uma vez que resulta em custos adicionais relacionados à saúde dos pacientes (3). Em abordagem síncrona com a Organização Mundial da Saúde (OMS), pioneira em destacar a urgência do assunto, e com o intuito do estabelecimento do conceito “One Health”, surgiu o Plano de Ação Nacional para Prevenção e Controle da Resistência aos Antimicrobianos do Brasil (PAN-BR). O objetivo geral dos planos de ação é o enfrentamento da conscientização e capacitação dos profissionais da saúde na escolha do antimicrobiano para o tratamento e prevenção das doenças, além da vigilância epidemiológica (4,5).

A resistência bacteriana a antibióticos ocorre quando bactérias são capazes de sobreviver e se multiplicar em concentrações de antibióticos que normalmente deveriam eliminá-las. Essa resistência se desenvolve ao longo do tempo devido à pressão seletiva exercida pelos antimicrobianos. Em trabalho, Varela et al., (2021) citam que os mecanismos bacterianos de resistência a antibióticos incluem: hidrólise enzimática, modificações no alvo de ação do fármaco, redução de permeabilidade da membrana por alterações em porinas e por bombas de efluxo. O uso inadequado de antimicrobianos aumenta a propagação de genes

de resistência para além dos microrganismos causadores de doenças, limitando as opções de tratamento disponíveis (7).

A proximidade homem e animal e o possível compartilhamento de água e alimentos contaminados propiciam a ocorrência de zoonoses e a viável disseminação de genes de resistência na comunidade, acarretando em riscos à saúde pública (8,9).

1.2 *Staphylococcus* Meticilina-Resistentes

Os *Staphylococcus* estão vastamente distribuídos e adaptados em sobreviver no meio ambiente e no microbioma dos humanos e animais. Existem diferentes espécies de *Staphylococcus* que apresentam variações de prevalência de acordo com a espécie e estado de saúde dos hospedeiros (10). Esses microrganismos podem ser categorizados em coagulase-positivos e coagulase-negativos, sendo a produção da enzima coagulase a principal distinção entre eles. A coagulase é uma enzima que converte o fibrinogênio do soro sanguíneo em fibrina, promovendo a coagulação. A espécie estafilocócica mais conhecida pela produção de coagulase e associada à infecções é o *S.aureus*.

A resistência dos *Staphylococcus* aos antibióticos da classe dos β-lactâmicos é atribuída principalmente a dois mecanismos distintos. O primeiro refere-se à produção da enzima β-lactamase, que hidrolisa o anel β-lactâmico dessa classe de antibiótico (11,12). Para combater os *Staphylococcus* produtores de β-lactamases foram criadas as penicilinas semi-sintéticas, entre elas a meticilina, que possuem radicais que as protegem da ação das β-lactamases. Não demorou para constatação de cepas de *Staphylococcus* meticilina-resistente (MRS), comprovando a plasticidade do genoma desta bactéria e a capacidade em se adaptar à pressão seletiva dos antibióticos (13,14). O segundo mecanismo de resistência aos β-lactâmicos está associada à produção da proteína de ligação a penicilina

adicional, a PBP2a ou PBP', que são capazes de continuar a síntese da parede celular enquanto as PBP nativas estão inativas. As PB2a atuam como transpeptidases e mantm a integridade da célula bacteriana na presença dos antibióticos β-lactâmicos (15–17). Os *Staphylococcus* spp. que possui a PBP2a mostram baixa afinidade não só as penicilinas semi-sintéticas, mas a praticamente todos β-lactâmicos.

Os MRS tem se tornado uma das principais preocupações em saúde pública de âmbito mundial. Em um estudo abrangente com dados de 204 países e territórios, o *S. aureus*, juntamente com *E. coli*, *K. pneumoniae*, *S. pneumoniae*, *Acinetobacter baumannii* e *Pseudomonas aeruginosa*, foram identificados como agentes globalmente responsáveis por 929.000 mortes em 2019. O mesmo estudo também revelou que somente *Staphylococcus aureus* meticilina-resistente (MRSA) causou mais de 100.000 mortes atribuíveis à RAM no mesmo ano (18).

1.3 *Staphylococcus* Meticilina-Resistentes em Animais

Diversas espécies de *Staphylococcus* são de importância conhecidas tanto na Medicina humana como na veterinária. O primeiro relato reportado à literatura de MRSA em animais ocorreu em bovinos leiteiros com mastite, nos anos 70 (19) e desde então, a presença desse microrganismos em animais está difundida em diversos países.

É sabido que os seres humanos desempenham papel relevante como hospedeiros intermediários para disseminação de MRS entre diferentes espécies animais (9,20), porém esses microrganismos têm sido cada vez mais identificados em diversos animais vertebrados e têm mostrado várias trocas de hospedeiros ao longo da evolução (16,21,22). Informações estas que corroboram com Richardson et al. (2018), que citaram a presença de genes responsáveis pela RAM em hospedeiros humanos e animais de forma difusa.

Recentemente, os animais domesticados têm sido apontados como reservatórios principais de cepas de MRS envolvidos em doenças humanas.

Diante da capacidade já mencionada de adaptação dos *Staphylococcus* spp., estes microrganismos são responsáveis por diversos tipos de infecções em animais, das mais brandas até fatais, incluindo em trato urinário, dermatites, mastites, pneumonias e septicemias (8,9). Tanto os animais quanto seus tutores podem ser colonizados por MRS como resultado da interação diária indireta entre eles e também pelo compartilhamento de ambiente e utensílios domésticos (20).

A forma de manifestação dos *Staphylococcus* spp. depende de diversos fatores de interação, entre eles: a imunidade do hospedeiro e capacidade de resistência a antibióticos (13).

1.4 Antibióticos β-lactâmicos

Antibióticos desse grupo recebem essa denominação por possuírem em sua estrutura química um anel β-lactâmico, o qual possui ação antimicrobiana e podem ser classificados em penicilinas, cefalosporinas, carbapenêmicos e monobactâmicos (24).

A caracterização das classes distintas de antibióticos beta-lactâmicos está relacionada à ligação do anel β-lactâmico com diferentes anéis, como o anel tiazolidínico nas penicilinas ou o anel di-hidrotiazina nas cefalosporinas. Em contrapartida, os carbapenêmicos possuem uma estrutura de anel duplo, enquanto os monobactâmicos não apresentam anéis adicionais (25).

O mecanismo de ação desses antibióticos ocorre devido à semelhança estrutural dos β-lactâmicos e do dípeptídeo D-Ala-D-Ala que compõe o peptidoglicano bacteriano. Como resultado, eles funcionam como inibidores da síntese da parede celular da bactéria (24).

Os antibióticos β-lactâmicos têm como alvo a etapa de biossíntese do peptidoglicano, a qual é catalisada pelas enzimas conhecidas como transpeptidases. Essas enzimas, conhecidas como proteínas de ligação à penicilina (PBPs), são encontradas na maioria das espécies bacterianas, geralmente, em pelo menos quatro formas distintas. A ação dos antibióticos β-lactâmicos consiste na inibição efetiva da atividade catalítica das transpeptidases bacterianas (24,26). Esse processo ocorre ao se ligarem às PBPs, o que impede o transporte de peptidoglicanos essenciais para a formação da parede dos microrganismos e, como resultado, prejudica a replicação bacteriana, levando à lise celular e morte (27).

Essa classe de antibióticos são amplamente utilizadas tanto na Medicina humana, como veterinária. A ausência de peptidoglicanos e transpeptidases nas células eucarióticas permite o uso seguro de antibióticos β-lactâmicos em animais e humanos, devido à sua eficácia terapêutica e baixa toxicidade (20,28).

Objetivos

1. Detectar a ocorrência de *Staphylococcus* meticilina-resistente (MRS) em animais atendidos em clínicas veterinárias da região de São José do Rio Preto, São Paulo, Brasil;

2. Identificar dentre os *Staphylococcus*, os grupos mais presentes nas amostras biológicas, no período de janeiro de 2019 à junho 2022;

3. Investigar as características fenotípicas de resistência dos isolados aos antimicrobianos avaliados em teste de antibiograma.

2.ARTIGO CIENTÍFICO

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Os resultados desta tese estão apresentados na forma de artigo submetido à publicação.

ARTIGO

Título: Ocurrance of Methicillin-Resistant *Staphylococcus* spp. in Animals Treated at Veterinary Clinics in the Northwest of the State of São Paulo, Brazil

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ARTIGO CIENTÍFICO

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Occurrence of Methicillin-Resistant *Staphylococcus* spp. in Animals Treated at Veterinary Clinics in the Northwest of the State of São Paulo, Brazil

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Abstract

Bacterial resistance is aggravated by inappropriate use of antibiotics, which amplifies the spread of resistance genes beyond disease-causing microorganisms, which ends up severely limiting the available treatment options, exposing public health risks. This practice increases the spread of resistance genes beyond disease-causing microorganisms, thereby severely reducing available treatment options. Staphylococci are adapted to survive both in the environment and in the microbiome of humans and animals. The objective of this study was to investigate the occurrence of methicillin-resistant *Staphylococcus* (MRS) in animals treated in veterinary clinics, observe the most common groups of these microorganisms in infections and verify the phenotypic characteristics of resistance of the isolates to antimicrobials in the antibiogram. This study used data from culture reports and antibiograms of biological samples from animals treated at veterinary clinics. This is a retrospective documentary study, with a mainly quantitative approach. Only reports indicating the presence of MRS were considered. The study sample included 118 cases, of which 94 were dogs (79.7%), 18 cats (15.3%), one bird (0.8%), two cattle (1. 7%), two horses (1.7%) and one lagomorph (0.8%). Of the MRS isolates analyzed, 73 (61.9%) were coagulase-negative, 25 (21.2%) *Staphylococcus aureus* and 20 (16.9%) of other coagulase-positive species. The auditory canal was the most common source of MRS-positive specimens (28.8%). The bacteria examined in this study exhibited notable resistance to β-lactams, as well as to other drug classes such as macrolides, clindamycin and quinolones. Constant monitoring of bacterial resistance is crucial to understand resistance patterns and reduce public health risks.

Key words: Coagulase-negative *Staphylococcus*; Coagulase-positive *Staphylococcus*; Methicillin-resistant *Staphylococcus aureus*, Domestic animals; Bacterial resistance; β-lactams

Introduction

The increase and spread of antimicrobial-resistant microorganisms are a worldwide concern in both human and veterinary medicine. In Brazil, despite the great concern about antimicrobial resistance (AMR) in companion animals and food producers, as well as the implications for public health, there is still a need for epidemiological information on microorganisms that transit and act outside the hospital environment (Melo et al., 2018; BrCAST, 2023).

The synchronous approach with the World Health Organization (WHO), a pioneer in highlighting the urgency of this theme, and with the aim of establishing the 'One Health' concept in Brazil, resulted in the National Action Plan for the Prevention and Control of Antimicrobial Resistance (PAN-BR). The general objective of this action plan is to address awareness and training of healthcare professionals in the choice of antimicrobials for the treatment and prevention of diseases, in addition to epidemiological surveillance in this regard (BRASIL, 2019; Caetano et al., 2022).

Bacterial resistance is aggravated by inappropriate use of antibiotics, which amplifies the spread of resistance genes beyond disease-causing microorganisms, which ends up severely limiting the available treatment options (WHO, 2021). The proximity of man and animal and the possible sharing of contaminated water and food favor the occurrence of zoonoses and enables dissemination of resistance genes in the community, resulting in public health risks (Argudín et al., 2017; Haag et al., 2019).

Staphylococci are widely distributed and adapted to survive in the environment and in the microbiome of humans and animals. *Staphylococcus* bacteria vary in prevalence according to the species and the health status of the host (Abdelmalek et al., 2022); they can be grouped as coagulase-positive and coagulase-negative. Richardson et al. (2018), reported that although the genes responsible for ARM are diffusely present in human and animal hosts, animals have recently been identified as the main reservoirs of *Staphylococcus aureus* strains involved in human diseases. These pathogens are responsible for several types of infections in animals, from mild to fatal, including dermatitis, mastitis, pneumonia and septicemia (Argudín et al., 2017). The manifestation of *Staphylococcus* depends on several interacting factors, including: host immunity and resistance to antibiotics (BRASIL, 2013).

In a comprehensive study using data from 204 countries and territories, *S. aureus*, along with *E. coli*, *K. pneumoniae*, *S. pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, were identified as agents globally responsible for 929,000 deaths in 2019. The same study also revealed that meticillin-resistant *S.aureus* (MRSA) alone caused more than 100,000 deaths attributable to AMR in the same year (Murray et al., 2022).

The Centers for Disease Control and Prevention (CDC) states that the cost of AMR to national economies and their health systems is significant, as it affects the productivity of patients or their caretakers through prolonged hospital stays and the need for more expensive and intensive care (CDC, 2021).

In this context, the present work aimed to detect the occurrence of methicillin-resistant *Staphylococcus* (MRS) in animals treated in veterinary clinics, identify among Staphylococcus the most common groups of these microorganisms in the biological

samples, and investigate the phenotypic characteristics of antimicrobial resistance of isolates as tested by antibiograms.

Materials and Methods

A retrospective, cross-sectional, documentary study with a descriptive character and a predominantly quantitative approach was carried out at the Veterinary Analysis and Pathology Center (CIAPAV), located in the city of São José do Rio Preto, São Paulo, Brazil. In this study, 4.793 culture reports and antibiograms of different biological materials from animals treated at veterinary clinics located in northwestern São Paulo State from January 2019 to June 2022 were evaluated. Only reports with proven presence of MRS were included in the investigation, totaling 118. The dependent variables considered in this study were the species of animal, the biological type or tissue sent to the laboratory, the bacteria isolated and the antimicrobial resistance profile. The independent variables in this study were the species of animal and the biological type or tissue sent to the laboratory. Information was collected from the physical reports provided by the aforementioned laboratory, which enabled the consolidation of data in an Excel spreadsheet for subsequent statistical analyses.

Phenotypic characterization of *Staphylococcus*

Bacteria were isolated from animal biological samples sent to the laboratory. After receiving the sample, the bacteria were cultivated according to the material received and bacterioscopy was performed followed by Gram staining to confirm the presence of cocci. Identification of the *Staphylococcus* phenotype followed ANVISA (BRASIL, 2013) guidelines by the inoculation in sheep blood agar and evaluation of the culture

characteristics. The catalase test and plasma coagulase test were used as methods of identifying *S. aureus*, coagulase-positive *Staphylococcus* (CoPS), coagulase-negative *Staphylococcus* (CoNS).

Antimicrobial susceptibility test

The susceptibility profile of *Staphylococcus* to antimicrobials was determined for 27 antibiotics by the disk diffusion method according to the BrCAST-EUCAST guidelines. The choice of antibiotics followed the laboratory's internal policy, which considered aspects such as the type of sample, the type of bacteria characterized and the site of action of the antibiotic. The antibiotics tested were oxacillin (1µg), amoxicillin (10µg), amoxicillin/clavulanic acid (30µg), ampicillin (10µg), ampicillin/sulbactam (20µg), cephalexin (30µg), cefadroxil (30µg), cefazolin (30µg), cefoxitin (30µg), ceftiofur (30µg), ceftriaxone (30µg), cefovecin (30µg), cefepime (30µg), meropenem (10µg), azithromycin (15µg), erythromycin (15µg), doxycycline (30µg), tetracycline (30µg), gentamicin (10µg), amikacin (30µg), neomycin (30µg), tobramycin (10µg), nitrofurantoin (30µg), chloramphenicol (30µg), florfenicol (30µg), vancomycin (30µg) and linezolid (10µg). The MRS disk diffusion detection method and the breakpoints were performed as established by BrCAST-EUCAST. Strains with zone sizes less than the described breakpoints were considered resistant to all classes of β-lactams. S. aureus ATCC 29213 was used as quality control.

Statistical analysis

Descriptive statistical analysis was performed based on calculations of measures of central tendency, dispersion and frequency. The chi-square test was used for inferential

statistical analysis. For all analyses, statistically significant values were defined when the p-value ≤ 0.05. The SPSS (IBM®, version 23, 2014) and GraphPad Instat (3.10, 2009) software were employed.

Results

Of the 4793 culture and antibiogram reports evaluated, 118 specimens were MRS positive resulting in a prevalence of 2.46%. In the present study, MRS infected six different animal species: of the 118 samples, positive cases included 94 dogs (79.7%), 18 cats (15.3%), one bird (0.8%), two cattle (1.7%), two horses (1.7%) and one lagomorph (0.8%), as shown in Table 1.

Table 1: Absolute and relative frequencies of MRS isolates of by animal species

Animal species	Absolute frequency	Relative frequency (%)
Bird	1	0.8
Cattle	2	1.7
Horse	2	1.7
Dog	94	79.7
Cat	18	15.3
Lagomorph	1	0.8
Total	118	100

Of the total number of MRS isolates identified in samples processed in the laboratory, 73 (61.9%) were methicillin-resistant coagulase-negative *Staphylococcus* (MRCoNS), 25

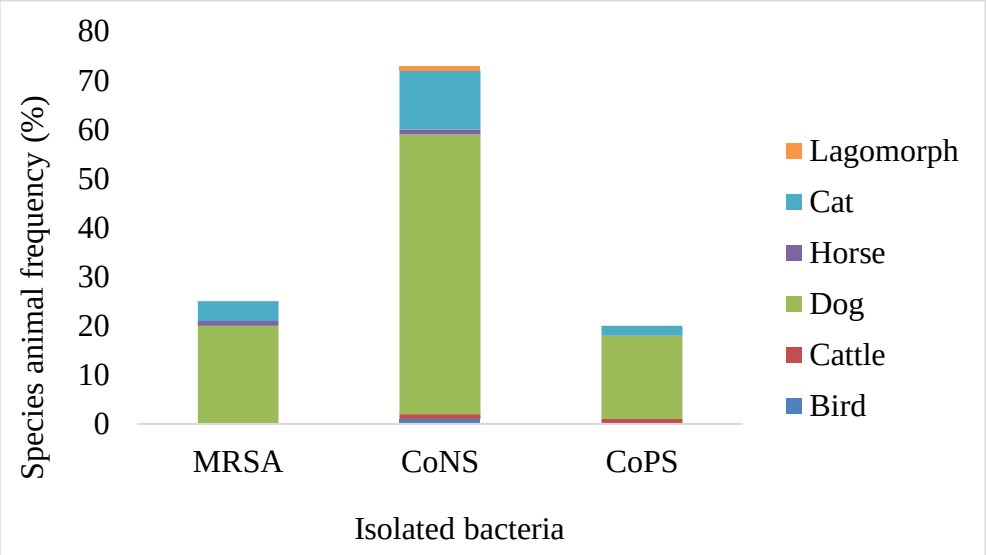
(21.2%) were MRSA and 20 (16.9%) were other species of methicillin-resistant coagulase-positive *Staphylococcus* (MRCoPS) (Table 2).

Table 2: Absolute and relative frequencies of MRS-positive groups isolated from biological samples

Staphylococcus	**Absolute frequency**	**Relative frequency (%)**
MRSA	25	21.2
MRCoNS	73	61.9
MRCoPS	20	16.9
Total	118	100

Among the animals infected by MRCoNS in this study were 57 dogs (78.1%), 12 cats (16.4%), one bird (1.4%), one cattle (1.4%), one horse (1.4%) and one lagomorph (1.4%). Of the samples in which MRCoPS were identified, 85% were from dogs (n = 17), 10% from cats (n = 2) and 5% from cattle (n = 1). Moreover, 80% of the MRSA-positive animal samples were from dogs (n = 20), 16% from cats (n = 4) and 4% from horses (n = 1 - Figure 1). There was no relationship between the involved animal species and the occurrence of MRS (p-value > 0.05).

Figure 1: Absolute frequencies of methicillin-resistant *Staphylococcus* isolated from different animal species



MRS were present in animal biological specimens from different sources, with the auditory canal (28.8%) being the most common, followed by skin lesions (24.6%) and the sum of all the unidentified clinical samples sent to the laboratory (19.5% - Table 3). Resistant bacteria were also found in urine (6.7%), the nasal cavity (5.8%), bone (3.3%), vaginal secretions (2.5%) and mammary secretions (2.5%). The most common microorganisms from all the different sample sources were MRCoNS, except for bone, which had a higher MRSA count (2.5%). A total of 21.2% of the samples were MRSA, with the highest occurance being identified auditory canal (7.6%). In the current study, low occurrences of MRS were found in specimens from surgical apparatus (0.8%), ocular secretions (1.6%) and ovarian secretions (0.8%), and abdominal (0.8%), oral (0.8%) and thoracic cavities (0.8%). No statistically significant correlation was found between the

frequencies of the bacteria isolated in samples from different sources (p-value > 0.05), indicating that the type of *Staphylococcus* is not related to the source.

Table 3: Absolute frequencies of MRS isolates in biological samples from different sources

	Sample content														
***Staphylococcus* type**	Surgical apparatus	Auditory canal	Abdominal cavity	Nasal cavity	Oral cavity	Thoracic cavity	Mammary secretions	Skin lesions	N.I.	Bone	Ocular secretions	Ovarian secretions	Vaginal secretions	Urine	Total
MRSA	1	9	0	1	0	0	0	4	4	3	1	0	1	1	25
MRCoNS	0	18	1	5	1	1	2	20	16	1	1	1	2	4	73
MRCoPS	0	7	0	1	0	0	1	5	3	0	0	0	0	3	20
Total	1	34	1	7	1	1	3	29	23	4	2	1	3	8	118

N.I.: not informed

All microorganisms tested with the β-lactam classes displayed high resistance to these antimicrobials. The association with a β-lactamase inhibitor (clavulanic acid) seemed to exert a greater action on MRSA microorganisms (92%; 23/25) compared to all the β-lactams alone (Table 4), but still with little effect. A similar result was observed for meropenem (85.7%; 6/7). Meropenem belongs to the class of carbapenems and has an inhibitory effect on bacterial cell wall synthesis. The other types of MRCoPS also exhibited resistance to meropenem (33.3%; 1/3).

Table 4: Absolute and relative frequencies of phenotypic resistance profiles of the MRS isolated

	S. aureus		**CoNS**		**CoPS**	
Antimicrobial agent	A.F.	R.F.	A.F.	R.F.	A.F.	R.F.
Oxacillin	25	100.0%	73	100.0%	20	100.0%
Amoxicillin/Clavulanic acid	25	92.0%	73	93.2%	20	100.0%
Amoxicillin	4	100.0%	10	100.0%	0	0.0%
Ampicillin	3	100.0%	11	100.0%	3	100.0%
Ampicillin/sulbactam	0	0.0%	3	66.7%	0	0.0%
Cephalexin	23	100.0%	69	98.6%	17	100.0%
Cefadroxil	20	100.0%	46	97.8%	11	100.0%
Cefazolin	0	0.0%	3	100.0%	1	100.0%
Cefoxitin	2	100.0%	12	66.7%	0	0.0%
Ceftiofur	4	100.0%	7	85.7%	1	100.0%
Ceftriaxone	15	100.0%	30	90.0%	6	100.0%
Cefovecin	12	100.0%	49	85.7%	11	81.8%
Cefepime	1	100.0%	3	100.0%	0	0.0%
Meropenem	7	85.7%	17	76.5%	3	33.3%
Azithromycin	22	81.8%	58	63.8%	17	76.5%
Erythromycin	19	89.5%	50	72.0%	16	100.0%
Clindamycin	19	84.2%	54	77.8%	14	92.9%
Ciprofloxacin	24	70.8%	71	59.2%	19	73.7%
Enrofloxacin	24	70.8%	71	64.8%	18	72.2%
Marbofloxacin	24	66.7%	69	65.2%	19	73.7%

Ofloxacin	12	75.0%	32	78.1%	10	80.0%
Levofloxacin	13	76.9%	21	47.6%	6	66.7%
Sulfamethoxazole/Trimethoprim	23	78.3%	66	84.8%	19	78.9%
Doxycycline	22	72.7%	57	63.2%	14	42.9%
Tetracycline	11	63.6%	20	80.0%	3	33.3%
Gentamicin	24	58.3%	69	62.3%	18	38.9%
Amikacin	22	9.1%	65	9.2%	17	5.9%
Neomycin	18	50.0%	41	65.9%	10	70.0%
Tobramycin	12	50.0%	42	52.4%	11	54.5%
Nitrofurantoin	2	50.0%	6	50.0%	2	0.0%
Chloramphenicol	13	30.8%	42	40.5%	10	30.0%
Florfenicol	11	9.1%	20	10.0%	6	0.0%
Vancomycin	9	22.2%	16	43.8%	3	33.3%
Linezolid	8	12.5%	13	15.4%	0	0.0%

A.F..: Absolute frequency; R.F.: Relative frequency

Of the MRCoNS, resistance was observed for compounds associated with β-lactamase inhibitors, such as clavulanic acid (93.2%; 68/73) and sulbactam (66.7%; 2/3). High resistance was found for MRCoNS tested with meropenem (76.5%, 13/17).

In general, the evaluated bacteria were highly resistance to the macrolides tested. Among the antibiotics to which the MRSA showed high resistance, in addition to the β-lactams, including third- and fourth-generation cephalosporins, were macrolides (erythromycin 17/19; azithromycin 17/22) and clindamycin (15/19), with all having percentages above 80%. MRSA showed resistance rates similar to those previously cited for quinolones:

ciprofloxacin (70.8%; 17/24), enrofloxacin (70.8%; 17/24), ofloxacin (75%; 9/12) and levofloxacin (76.9%; 10/13). Moderate resistance was seen in respect to aminoglycosides and nitrofurans for these microorganisms. The MRSA in this study showed a high degree of resistance with regard to sulfonamide/trimethoprim and doxycycline.

A very similar profile was characterized for the MRCoPS, with greater differences for tetracycline (33.3%; 1/3) and doxycycline (42.9%; 6/14), to which the microorganisms showed less resistance (Table 4). However, resistance to erythromycin (100%, 16/16) and clindamycin (92.9%; 13/14) was significant for these bacteria. Furthermore, MRCoPS showed less resistance to aminoglycosides, with the exception of neomycin (70%; 7/10) and tobramycin (54.5%; 6/11).

Most quinolones, aminoglycosides and doxycycline showed intermediate efficacy against MRCoNS, with an average of 60% resistance. Among this group of *Staphylococcus*, there was greater resistance to chloramphenicol (40.5%; 17/42) in relation to the previously mentioned group.

Isolates resistant to vancomycin were identified in all groups of *Staphylococcus* with MRCoNS being the most resistant (43.8%; 7/16). Linezolid was tested on a few isolates, and even so, resistance was found for MRSA (12.5%; 1/8) and MRCoNS (15.4%; 2/13).

Of the antibiotics tested, the aminoglycoside, amikacin showed the greatest efficiency against the bacteria identified in this research, followed by the amphenicols, vancomycin and linezolid.

Discussion

This study showed that MRS were more common in dogs and cats. Similar results have been reported in several works, along with the growing concern in relation to resistant

bacteria in domestic animals and their implications on animal and public health (Loncaric et al., 2019). The greater observation of resistant bacteria in dogs and cats seems to be linked to the proximity of these species to humans (Haag et al., 2019; Costa et al., 2022). The figures described for MRS may not demonstrate the real situation for birds, cattle, horses and lagomorphs, as in this study the occurrence of these species was low possibly because these animals fall in categories for which investigation of infections is remiss. In addition, the presence of MRS in dogs and cats can be justified, in part, by the greater number of treatments with antimicrobials they receive throughout their lives compared to other species.

Although CoPS are reported as the main causal agents of skin and soft tissue infections in dogs and cats (Costa et al., 2022), this was not observed in this study, which found more MRCoNS infections. These results corroborate those described by Loncaric et al. (2019), who commented on the scarcity of available data on this group of resistant bacteria in animals. The CoNS most commonly associated with animal and human infections are *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. warneri* and *S. schleiferi* (Mombach Pinheiro Machado et al., 2007; Loncaric et al., 2019; Teixeira et al., 2019). In contrast to the present study, of the 43 MRS strains characterized in the work by Bzdil et al. (2021) on sick dogs and cats from the Czech Republic, the most prevalent species were the coagulase positive *S. pseudintermedius* (n = 24; 0.46%) and *S. aureus* (n = 7; 0.13%). With regard to the MRCoPS in this study, most of the strains of this group of bacteria were MRSA found in specimens from the auditory canal. *S. aureus*, which are frequently found in dogs and cats, can act as an opportunistic pathogen causing various clinical conditions. Several works describe the prevalence of MRSA in diseases of different

animals, including mammary and uterine infections, osteomyelitis, pyoderma, etc. (Yadav et al., 2018; Bzdil et al., 2021; Bellato et al., 2022; Costa et al., 2022).

A relevant aspect to mention is that the veterinarian requesting the examination did not identify the sources of 19.5% of the MRS-positive samples sent to the laboratory. It is essential to provide more detail on the patient, which in addition to ensuring the correct processing of the sample, allows the data to be used in epidemiological research. Thirty-four antibiotics were evaluated using the disk diffusion technique with Müeller-Hinton agar, following the BrCAST-EUCAST guidelines to determine antimicrobial susceptibility of bacteria isolated from animals. Resistance was observed for all classes of antibiotics studied. All microorganisms tested with β-lactams showed resistance. It was found that antibiotics that contained β-lactamase inhibitors (clavulanic acid and sulbactam) had a little better inhibitory effect on MRSA and MRCoNS compared to β-lactams without these compounds. In an investigation in the northern region of Minas Gerais into the frequency of *S. aureus* in subclinical bovine mastitis that determined susceptibility of these bacteria to β-lactams, it was noted that of the 79 specimens, on average 36.3% were resistant to cefoxitin, oxacillin and amoxicil, only 11 .4% were resistant to meropenem and none to ampicillin with sulbactam (Souza et al., 2020). In comparison, the isolates in our study showed a greater resistance to the compound meropenem. The bacteria that most produce carbapenemases are reportedly from the Enterobacteriaceae group, which have a high potential to transmit resistance genes (WHO, 2017). The continuous use of β-lactams may explain resistance to meropenem by Staphylococci, since the production of carbapenemases is not considered the prevalent mechanism of resistance and selective pressure can lead to an increase in the occurrence of multiple β-lactamases in a single organism (Bush and Bradford, 2020). Another aspect

is that MRSA carriers of the mecA gene can be resistant to all penicillins, cephalosporins and carbapenems (Silva et al., 2020).

Most strains showed similar resistance to macrolides (erythromycin, azithromycin), clindamycin and quinolones. In evaluating the susceptibility profile of *S. aureus* isolated from companion animals in Portugal, Costa et al. (2022) noted a high prevalence of resistance to quinolones, but not to clindamycin and erythromycin. Information such as this demonstrates that the genes and mechanisms involved in bacterial resistance may vary according to the geographic location, mainly due to the pattern of prescription of antibiotics in each region.

Of the antibiotics tested for MRCoNS isolated from asymptomatic dogs with otitis and pyoderma in a study conducted in Brazil, it was observed that the bacteria were less susceptible to erythromycin, tetracycline and clindamycin and that all of the 17 isolates were susceptible to nitrofurantoin (Teixeira et al., 2019). Bacteria from the same group in our study showed a very similar and more expressive resistance profile for these antibiotics, with the exception of nitrofurantoin, which was effective in only half of the tested isolates.

In the face of MRS isolation, the main treatment has become the use of the drug vancomycin, a glycopeptide. Strains of *Staphylococcus* spp. with intermediate or complete resistance to vancomycin have been reported in the literature in recent years (Moreira et al., 2020; Abdallah et al., 2022b). Of the 28 isolates tested, ten were resistant to vancomycin. Of the 29 MRSA found using nasal, ear and skin swabs from sick and healthy dogs in a research carried out by Abdallah et al. (2022), all were resistant to vancomycin and susceptible to ciprofloxacin. In the current study, the microorganisms most tested with vancomycin were coagulase-negative *Staphylococcus*, which were

proportionally also the most resistant. MRCoPS and MRCoNS strains resistant to vancomycin were found in domestic dogs in Paraná, demonstrating the importance of monitoring the resistance profiles of bacteria circulating in the community (Santos et al., 2022).

Another point that deserves attention is the prevalence of bacteria resistant to certain antibiotics in different geographic regions. This is explained in a study conducted in Nigeria, where MRSA isolated from dogs were all susceptible to vancomycin and linezolid in a country where these drugs are not commonly used in animal and human treatment (Emele and Chikezie, 2019). There were three resistant isolates among the 22 tested for the antibiotic linezolid, two MRCoNS and one MRSA. Resistance to vancomycin and linezolid in animals has already been described, however the literature on this subject is more focused on research in humans (Moawad et al., 2019; Shoaib, 2020; Gostev et al., 2021; Timmermans et al., 2021; Aqib and Alsayeqh, 2022). The increased use of these antibiotics, or the use of long cycles of therapy, causes conditions of unpredictable bacterial resistance. In view of this, failure in epidemiological pursuit and tracking of resistant bacteria in animals is a worrying situation due to the intimate coexistence between man and animals.

Conclusion

In conclusion, the present study reports a higher occurence of MRS in dogs and cats, since most of the analyzed biological samples were collected from these animal species. The highest observations of bacteria, especially MRCoNS, came from the ear canal and skin lesions which highlights the need to track other resistant *Staphylococcus* strains with research that goes beyond known microorganisms, such as *S. aureus*, involved in animal

infections. In general, the bacteria in this study showed notable frequencies of resistance to β-lactams and other antibiotic classes such as the macrolides, clindamycin and quinolones. Vancomycin-resistant isolates were found in all groups of *Staphylococcus*. There is a gap in the availability of specific epidemiological information on resistant microorganisms in domestic animals. Continuous surveillance of bacterial resistance in animals is essential to prevent risk to human health and for decision making in the clinical practice.

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3. CONCLUSÃO

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ARTIGO CIENTÍFICO

1. Maior ocorrência de MRS em cães e gatos devido à maioria das amostras analisadas advirem dessas espécies animais.

2. Maior observação de bactérias em conduto auditivo e lesão cutânea com MRCoNS, destacando a necessidade de rastreamento de outras cepas de *Staphylococcus* resistentes.

3. Notáveis níveis de resistência bacteriana às classes de antibióticos β-lactâmicos, macrolídeos, clindamicina e quinolonas. Houve isolados resistentes à vancomicina em todos os grupos de *Staphylococcus*.

4. Este estudo é o primeiro na região de São José do Rio Preto, São Paulo, Brasil, a abordar essa preocupação epidemiológica. São necessárias mais pesquisas sobre o tema, uma vez que a resistência a múltiplos antibióticos representa uma ameaça global e emergente à saúde. Existe uma carência de informações epidemiológicas específicas sobre microrganismos resistentes em animais domésticos, destacando a importância da vigilância contínua para prevenir riscos à saúde humana e orientar a prática clínica.

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5. ANEXOS

Anexo I. Submissões de artigos

VETMIC-D-23-00728 - Confirming your submission to Veterinary Microbiology

Veterinary Microbiology <em@editorialmanager.com>
Sex, 18/08/2023 20:47
Para:Isabela Belei Delmaschio de Oliveira <isabelabelei@hotmail.com>

This is an automated message.

Prevalence of Methicillin-Resistant Staphylococcus spp. in Animals Treated at Veterinary Clinics in the Northwest of the State of São Paulo, Brazil

Dear Veterinarian Delmaschio de Oliveira,

We have received the above referenced manuscript you submitted to Veterinary Microbiology. It has been assigned the following manuscript number: VETMIC-D-23-00728.

To track the status of your manuscript, please log in as an author at https://www.editorialmanager.com/vetmic/, and navigate to the "Submissions Being Processed" folder.

Thank you for submitting your work to this journal.

Kind regards,
Veterinary Microbiology

Journal: Veterinary Microbiology
Title: Prevalence of Extended Spectrum β-lactamases (ESBL)-Producing Bacteria in Animals Treated at Veterinary Clinics in the Northwest of the State of São Paulo, Brazil
Corresponding Author: Veterinarian Leonardo Sanches
Co-Authors: Isabela Belei Delmaschio de Oliveira; Giovanna Rossi Varallo; Luana Augusto Andrade; Cinara Cássia Brandão
Manuscript Number: VETMIC-D-23-00737

Dear Cinara Cássia Brandão,

The corresponding author Veterinarian Leonardo Sanches has listed you as a contributing author of the following submission via Elsevier's online submission system for Veterinary Microbiology.

Submission Title: Prevalence of Extended Spectrum β-lactamases (ESBL)-Producing Bacteria in Animals Treated at Veterinary Clinics in the Northwest of the State of São Paulo, Brazil

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DECLARAÇÃO

Declaramos para os devidos fins que o artigo intitulado "**In sickness and in health: the intestinal microbiome of dogs**", de autoria de Giovanna Rossi Varallo, Gabriela Marchiori Bueno, Cinara de Cassia Brandão, Leonardo Sanches, Isabela Belei Delmaschio de Oliveira, sob o número de protocolo **203612**, foi aceito para publicação na revista *Brazilian Journal of Veterinary Research and Animal Science* no Volume 60 (2023).

São Paulo, 29 de maio de 2023.

Atenciosamente,

Prof. Dr. Silvio Arruda Vasconcellos
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