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MICROBIOLOGY

# Vancomycin susceptibility profiles of *Staphylococcus* spp. isolates from domestic and wild animals

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**ABSTRACT**: *Staphylococcus spp. are bacteria involved in human and animal infections. They are resistant to antimicrobials and have become a major public health concern. In recent years, there has been a significant increase in methicillin-resistant Staphylococcus strains and vancomycin is the drug of choice for the treatment of such isolates. However, the minimum inhibitory concentration (MIC) of vancomycin necessary to combat this microorganism has been showing an increase. The aim of the present study was to determine the susceptibility profile of the Staphylococcus spp. of domestic and wild animals to vancomycin, using the microdilution in broth and E-test® techniques, as well as comparing the results of both tests. Of the 50 isolates tested, 47 (94 %) were sensitive to vancomycin in the microdilution and 43 (86 %) were sensitive to vancomycin in the E-test®. Seven (14 %) isolates had an intermediate result showing a risk to public health since the detection of these isolates may precede the occurrence of isolates resistant to vancomycin. In addition, the mecA gene was detected in 78 % of the tested samples. Six of the seven isolates with intermediate resistance to vancomycin were carriers of the mecA gene, showing that these isolates had a potential risk of becoming resistant. Thus, control measures must be taken to prevent the spread of these isolates with intermediate resistance and preserve the effectiveness of this antimicrobial for the treatment of infections caused by multiresistant Staphylococcus spp.*
**Key words**: *multiresistant bacteria, minimum inhibitory concentration, Staphylococcus spp., intermediate resistance to vancomycin; mecA.*

# Perfil de suscetibilidade à Vancomicina de isolados de *Staphylococcus* spp. de animais domésticos e silvestres

**RESUMO**: *Staphylococcus spp. são bactérias envolvidas em infecções de humanos e animais, resistentes a antimicrobianos e tem se tornado uma grande preocupação em saúde pública. Nos últimos anos houve um aumento significativo de Staphylococcus resistentes à meticilina e a vancomicina é a droga de escolha para o tratamento desses isolados, porém vem apresentando elevação nos valores de Concentração Inibitória Mínima (CIM) necessários para combater este microrganismo. O objetivo do presente trabalho foi determinar o perfil de suscetibilidade à vancomicina para isolados de Staphylococcus spp. de animais domésticos e silvestres pelas técnicas de Microdiluição em caldo e E-test®, bem como comparar os resultados de ambos os testes. Dos 50 isolados testados 47 (94%) foram sensíveis à vancomicina na Microdiluição e 43 (86%) foram sensíveis à vancomicina no E-test®. Sete (14%) isolados tiveram resultado intermediário demonstrando um risco à saúde pública visto que a detecção destes isolados pode preceder a ocorrência de isolados resistentes à vancomicina. Ademais o gene mecA foi detectado em 78% das amostras testadas, sendo que dos sete isolados com resistência intermediária à vancomicina, seis eram portadores do gene mecA, evidenciando que esses isolados possuem potencial risco de se tornarem resistentes. Dessa forma medidas de controle devem ser tomadas para evitar a propagação destes isolados com resistência intermediária e preservar a eficácia deste antimicrobiano para o tratamento de infecções causadas por Staphylococcus multirresitentes.*
**Palavras-chave**: *bactérias multirresistentes, concentração inibitória mínima, Staphylococcus spp., resistência intermediária à vancomicina, mecA.*

## INTRODUCTION

Bacterial resistance to antibiotics is a threat to public health and drug-resistant bacteria could cause 10 million deaths each year until 2050. This problem has become a concern in veterinary medicine; the concept of "One Health", where human health, animal health and the environment are fully integrated, is extremely important when considering bacterial resistance to antimicrobials (ONU, 2019).

The potential risk of transmitting antimicrobial resistant isolates from animals to

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humans and vice-versa is associated with close contact between animals and their guardians and the inappropriate use of antimicrobials, since drugs used in the treatment of humans are administered in animals as growth enhancers and in the treatment and prophylaxis of infections (LOUREIRO et al., 2016; CERIC et al., 2019).

Bacteria of the genus *Staphylococcus* are considered opportunistic pathogens in several diseases of humans and animals, with *Staphylococcus aureus* being the species most commonly involved in human infections, followed by *S. pseudintermedius* in dogs and cats (SAPUTRA et al., 2017). The emergence of *Staphylococcus* spp. resistant to antimicrobials, especially methicillin, has been of concern in recent years, and effective treatment options in animals are decreasing (PAPICH, 2013).

Vancomycin is an antibiotic of the glycopeptide class, whose mechanism of action is to prevent the synthesis of the cell wall of microorganisms. In recent decades, it has been an effective solution in the treatment of infections caused by pathogens resistant to methicillin, particularly *Staphylococcus* spp. (MCGUINNESS et al., 2017). Among the glycopeptides, vancomycin is the only one used in veterinary medicine. Despite being a widely used drug in humans, its use in animals is still uncommon due to its toxicity, need for intravenous administration by slow infusion and its high cost. However, gradual increases in its minimum inhibitory concentration (MIC) values have been reported (PAPICH, 2013).

*Staphylococcus* spp. with reduced sensitivity to vancomycin has been reported in the last 20 years and isolates with intermediate resistance and hetero resistance are more frequent than isolates with full resistance (HUANG et al., 2016). The first strain of methicillin-resistant *Staphylococcus aureus* (MRSA) with intermediate resistance to vancomycin was reported by HIRAMATSU et al. (1997) in Japan, isolated from a child being treated with vancomycin due to a surgical infection. Although, *Staphylococcus* with reduced susceptibility to vancomycin has already been reported in animals (MORENO et al., 2016), there is still little research on the frequency of these isolates in animals (WIJESEKARA et al., 2017).

Thus, this research aimed to determine and compare the vancomycin MIC against isolates of *Staphylococcus* spp. from injuries to domestic and wild animals, by broth microdilution and E-test® techniques.

## MATERIALS AND METHODS

*Isolates*

The tested isolates of *Staphylococcus* spp. came from a previous study of the susceptibility profile to antimicrobials carried out by GODOY et al. (2016). One hundred isolates of *Staphylococcus* spp. underwent a vancomycin susceptibility test (30 µg, Cefar Diagnóstica Ltda), using the Mueller-Hinton agar diffusion disc method, according to BAUER et al. (1966), with an inhibition zone ≤ 14 mm. The isolates with a halo equal to or less than 14 mm were selected to determine the MIC of vancomycin, either by the broth microdilution method or by E-test®.

There were 50 isolates of *Staphylococcus* spp. altogether, 42 of which were isolated from dogs, 4 from felines, 1 from bovine, 1 from equine, 1 from *Procyon cancrivorus* (Raccoon), 1 from *Nasua nasua* (coati) and 1 from *Lycalopex vetulus* (fox of the field). All were isolated from clinical cases. These data are shown in table 1.

The isolates were previously identified as species of *Staphylococcus* by GODOY et al. (2016) and 92 % were coagulase positive, with 64 % (32/50) being *S. pseudintermedius* and 20 % (10/50) being *S. schleiferi*. *S. aureus* and *S. delphini* each represented only 4 % (2/50) of the total of isolates. Coagulase negative *Staphylococcus* (*S. sciuri*, *S. simulans* and *S. felis*) represented 8 % of the total samples (4/50).

*Minimum inhibitory concentration*

The broth microdilution technique is considered the gold standard technique and was performed according to CLSI (2013). Ten serial dilutions of vancomycin (Sigma-Aldrich), ranging from 64–0.125 µg/mL, were tested in Mueller-Hinton broth. After inoculation and standardization of the bacterial suspension to 0.5 on the McFarland scale followed by incubation at 37 °C for 24 h, the MIC was determined to be the lowest concentration in which there was no visible growth. The tests were performed in duplicate.

The E-test® (Biomerieux) was performed according to the manufacturer's instructions. After inoculation of the bacterial suspension (diluted to 0.5 on the McFarland scale) on Mueller-Hinton agar, the strip was deposited and incubated at 37 °C for 24 h. The reading was performed at the point where the ellipse intercepted the E-test® strip. *Enterococcus faecalis* ATCC 29212 (vancomycin-susceptible) and *Enterococcus faecalis* ATCC 51299 (vancomycin-resistant) were used as quality control of the tests.

Table 1 - Frequency of *Staphylococcus* species identified in different animal hosts.

| | | ----------Host---------- | | | | |
|---|---|---|---|---|---|---|
| Species | Injury site | Canine | Feline | Bovine | Equine | Wild |
| *S. aureus* | Feces | -- | -- | 1 | -- | -- |
| | injury swab | -- | -- | -- | 1 | -- |
| *S. delphini* | Otological swab | 1 | -- | -- | -- | -- |
| | Fur | 1 | -- | -- | -- | -- |
| *S. felis* | Otological swab | 1 | -- | -- | -- | -- |
| | Urine | -- | 1 | -- | -- | -- |
| *S. pseudintermedius* | Liquor | 1 | -- | -- | -- | -- |
| | Otological swab | 16 | 1 | -- | -- | 1c |
| | Fur | 6 | -- | -- | -- | -- |
| | Oral swab | -- | -- | -- | -- | 1[B] |
| | Eye swab | 2 | -- | -- | -- | -- |
| | Urine | 3 | 1 | -- | -- | -- |
| *S. schleiferi* | Otological swab | 9 | -- | -- | -- | -- |
| | Lung | 1 | -- | -- | -- | -- |
| *S. sciuri* | Eye swab | -- | -- | -- | -- | 1[A] |
| *S. simulans* | Nail | -- | 1 | -- | -- | -- |
| Total | | | | | | 50 |

[A] Fox : *Lycalopex vetulus*
[B] Raccoon: *Procyon cancrivorus*
[C] Coati: *Nasua nasua.*

*Interpretation of results*

Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) document VET01-S2, where isolates with MIC ≤ 4 µg/mL were considered sensitive, isolates with MIC between 8–16 µg/mL were considered intermediate and isolates with MIC ≥ 32 µg/mL were resistant (CLSI, 2013).

*Statistical analysis*

Statistical analysis of the data was performed using a Chi-squared test with a significance level of $P<0.05$. Correlation and the degree of agreement of the tests were measured by Kappa using R (R-3.6.1).

*Detection of the* mecA *gene*

Determination of methicillin resistance was also performed, by detecting the presence of the *mecA* gene through PCR, with specific primers *mecA*1 5'- CCT AGT AAA GCT CCG GAA -3 and *mecA*2 5'-CTA GTC CAT TCG GTC CA-3'. The primers amplify a 331 bp fragment of the *mecA* gene, according to the protocol described by CHOI et al. (2003). The amplification products were analysed by electrophoresis on 1.5 % agarose gel, stained with Gel Red ™ (Biotium®) and observed in a ChemiDoc ™ XRS photo-documenter using the ImageLab software, with a 100 bp molecular mass marker (Ludwig).

## RESULTS AND DISCUSSION

Of the 50 isolates tested for the determination of the MIC of vancomycin, 94 % (47/50) and 86 % (43/50) were sensitive to vancomycin by microdilution in broth and E-test® respectively. Fourteen percent (7/50) of the isolates showed intermediate resistance to vancomycin.

Several methodologies are used to determine the susceptibility profile to antimicrobials; however, for vancomycin it is recommended to determine the MIC by dilution methods (broth microdilution, tube macrodilution, water dilution) or, alternatively, by using E-test® tapes or automated systems (CLSI, 2013; CAMPANA et al., 2011).

The disk diffusion technique was used only as a screening method for *Staphylococcus* resistance to vancomycin, since it is not able to predict the MIC and is not recommended by CLSI (2013) due to the low accuracy in identifying isolates with intermediate or full resistance, and even isolates heteroresistant to this glycopeptide (CAMPANA et al., 2011).

It was reported that the vancomycin MICs varied from 1–8 µg/mL by microdilution in broth and from 1–16 µg/mL by E-test®. The majority of the MICs determined by the E-test® were 4 µg/mL (33 isolates, 66 %), while the majority of the MICs determined by microdilution were 2 µg/mL (33 isolates, 66 %). In figure 1, it is possible to see the distribution of MIC values for vancomycin obtained by the two tests. Results of the *Staphylococcus* spp. together, the MIC values for vancomycin by broth microdilution and E-test® can be seen in table 2.

PAIVA et al. (2010) reported MICs ranging from 0.25–2 µg/mL by microdilution and between 0.38–3 µg/mL by E-test® in a study with 130 coagulase-negative *Staphylococcus* spp. isolated from humans. SWENSSON et al. (2009), in a study with 129 *S. aureus* isolates, obtained MICs ranging from 0.5–8 µg/mL in both tests. The data demonstrated that the isolates tested in the present study had MICs greater than those reported by PAIVA et al. (2010) and similar to those reported by SWENSSON et al. (2009), evidencing the increase in vancomycin MICs needed to fight the microorganism.

Seven (14 %) of the isolates had intermediate resistance, three detected by the two tests and four detected only by the E-test®. Of these seven: five were *S. pseudintermedius* isolates from dogs, whose isolation sites were otological swab, fur and ocular swab; one was *S. aureus* isolated from bovine feces; and one was *S. schleiferi* isolated from a dog ear. The detection of these isolates is extremely important to establish a monitoring system for antimicrobial resistance with a "One Health" approach (CERIC et al., 2019) and represents a challenge for the treatment of infections caused by *Staphylococcus* spp. in humans and animals around the world as there is a need to avoid therapeutic failure (HUANG et al., 2016).

*Staphylococcus* spp. with methicillin resistance are considered multidrug-resistant (MDR) since they are resistant to all β-lactams and; therefore, are considered a challenge for clinical practice (CERIC et al., 2019). The *mecA* gene was detected in 78 % (39/50) of the samples tested in this study and six of the seven isolates with intermediate resistance to vancomycin were carriers of the *mecA* gene. This fact that may explain the detection of elevated vancomycin MIC values that resulted in isolates with intermediate resistance to glycopeptide, since infections by isolates resistant to methicillin

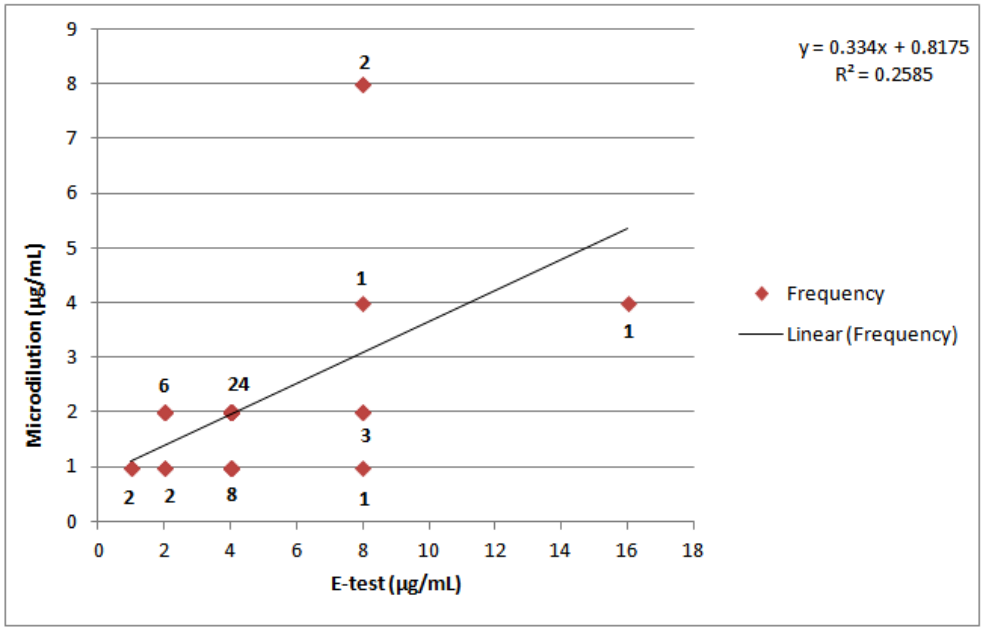


Figure 1 - Dispersion graph of the correlation between vancomycin MICs (µg/mL) determined by broth microdilution compared to the vancomycin MICs (µg/mL) determined by the E-test®.

constitute a risk factor associated with an elevated MIC of vancomycin (GOMES et al., 2015).

The significant increase in methicillin-resistant *Staphylococcus* spp. in recent years represents a risk not only for animals, but also for humans, since pet animals can participate in the cross-transmission of these multi-resistant isolates within the family environment (MORRIS et al., 2012). Allied to this, it has been reported that vancomycin shows low performance against *Staphylococcus* spp. resistant to methicillin that exhibit MICs close to the limit of susceptibility, demonstrating therapeutic failure (HUANG et al., 2016).

According to ROSSATO et al. (2014), the apparent increase in vancomycin MIC values between isolates of *Staphylococcus* spp. observed in recent years may represent the first step towards the occurrence of resistant isolates. In fact, the appearance of these strains, which are associated with therapeutic failure, was determined by the presentation of intermediate resistance or heteroresistance. Another factor responsible for the increase in MIC values of this antimicrobial is the use of vancomycin analogues such as avoparcin, which is used extensively as a growth enhancer in farm animals (WIJESEKARA et al., 2017).

In the present study, the isolates showed high susceptibility to vancomycin through the two tests used, which may be due to the low use of this antimicrobial in companion animals (SILVA et al., 2014). In a study of antimicrobial resistance in *S. pseudintermedius* isolated from dogs, RUZAUSKAS et al. (2016) observed that the isolates were only susceptible to antimicrobials of atypical use in veterinary medicine (vancomycin, linezolid and daptomycin), emphasising that such antimicrobials can only be used in the treatment of infections in humans to prevent the spread of resistance.

The detection of isolates with intermediate resistance varied according to the tests. The E-test® detected the largest number of isolates (n = 7) compared to the microdilution (n = 3), with

Table 2 - Distribution of minimum inhibitory concentration (MIC) values for vancomycin obtained by broth microdilution and E-test® methods according to the *Staphylococcus* species isolated from animals.

| Species | Broth microdilution / E-test MIC (µg / mL) | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 4 | 8 | 16 |
| *S. aureus* | | | | | |
| Microdilution | 0 | 1 | 1 | 0 | 0 |
| E-test | 0 | 0 | 1 | 1 | 0 |
| *S. delphini* | | | | | |
| Microdilution | 0 | 2 | 0 | 0 | 0 |
| E-test | 0 | 0 | 2 | 0 | 0 |
| *S. felis* | | | | | |
| Microdilution | 0 | 2 | 0 | 0 | 0 |
| E-test | 0 | 2 | 0 | 0 | 0 |
| *S. pseudintermedius* | | | | | |
| Microdilution | 12 | 18 | 0 | 2 | 0 |
| E-test | 2 | 3 | 22 | 4 | 1 |
| *S. scheliferi* | | | | | |
| Microdilution | 0 | 9 | 0 | 1 | 0 |
| E-test | 0 | 0 | 7 | 1 | 0 |
| *S. sciuri* | | | | | |
| Microdilution | 0 | 1 | 0 | 0 | 0 |
| E-test | 0 | 0 | 1 | 0 | 0 |
| *S. simulans* | | | | | |
| Microdilution | 1 | 0 | 0 | 0 | 0 |
| E-test | 0 | 1 | 0 | 0 | 0 |
| Total | | | | | |
| Microdilution | 13 | 33 | 1 | 3 | 0 |
| E-test | 2 | 8 | 33 | 6 | 1 |

three of the isolates being identified in both tests. This is probably due to the differences in the MIC detection of this antimicrobial by these techniques. Although, the E-test® is very effective in determining the vancomycin MIC, it is advisable that broth dilution methodologies are also used for strains with MICs close to the susceptibility cut-off point (MANFREDINI et al., 2011) since the E-test® tends to provide higher MIC results of vancomycin than microdilution in broth, resulting in a greater number of intermediate isolates (SWENSSON et al., 2009; PAIVA et al., 2010; MANFREDINI et al., 2011).

MORENO et al. (2016) reported the first methicillin-resistant *S. aureus* vancomycin-intermediate associated with livestock in Brazil (LA-MRSA ST398 / t9538), isolated from pigs with exudative epidermitis. The ST398 clone has been associated with livestock, especially pig farming, but it has also been linked to infections in humans, including patients with cystic fibrosis (LIMA et al., 2017). The identification of this MDR isolate warns of the high risk to public health and highlighted the need for further studies and surveillance actions on these microorganisms.

Companion and production animals are constantly implicated as potential reservoirs of *Staphylococcus* spp. with reduced sensitivity to antimicrobials, representing a potential risk to public health. Infections in humans by these microorganisms have already been reported, mainly by *S. pseudintermedius*, which is not part of the human microbiota and is believed to have a zoonotic character (PRIYANTHA et al., 2016, LOZANO et al., 2017, KMIECIAK & SZEWCZYK, 2018).

Thus, control measures with a "One Health" approach must be taken, including the prudent and correct use of antimicrobials in veterinary medicine to preserve the effectiveness of drugs for both animal and human use, as well as surveillance actions to decrease the number of MDR bacteria (CERIC et al., 2019).

Statistically significant associations ($P < 0.05$) were observed between the isolated *Staphylococcus* spp. (*S. pseudintermedius*) and the host (canine), as well between the *Staphylococcus* spp. (*S. pseudintermedius*) and the isolation site (ear). These associations were possibly due to the greater amount of samples obtained from canine otitis.

Only four isolates had conflicting results in the two tests (intermediate in the E-test and sensitive in the microdilution in broth): *S. pseudintermedius* isolated from eye swab, dog ear and fur; and *S. aureus* isolated from bovine faeces. The Kappa test agreement obtained a value of 0.56, which, according to LANDIS & KOCH (1977), gives a substantial agreement between them.

In conclusion, this is the first study on the susceptibility profile of *Staphylococcus* spp. isolated from domestic and wild animals to vancomycin in Mato Grosso, Brazil. Our results revealed an increase in the MIC values of vancomycin by broth microdilution and E-test® techniques, with the presence of seven *Staphylococcus* spp. isolates being detected with intermediate resistance to vancomycin. This is aggravated by the fact that 78 % of the tested isolates have the *mecA* gene, thus representing a potential risk to public health and the spread of these microorganisms.

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## DECLARATION OF CONFLICT OF INTERESTS

The authors declare that there are no potential conflicts of interest regarding the research, authorship and/or publication of this article.

## AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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