



Antimicrobial resistance and prevalence of *Staphylococcus aureus* in veterinary sources in South Brazil, 2017–2023

Resistência e prevalência de *Staphylococcus aureus* na região Sul do Brasil, 2017-2023

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DOI: 10.4025/revcivet.v12i1.70642

ABSTRACT

Surveillance studies are essential for tracking the emergence and spread of antimicrobial resistance, which can help identify the most effective strategies for international stewardship in containing the spread of resistant infections. This study investigated the prevalence and resistance patterns of *Staphylococcus aureus* in southern Brazil from 2017 to 2023. A total of 3,435 clinical specimens were collected from veterinary sources and evaluated for *Staphylococcus aureus*. Antibiotic susceptibility testing was performed using the disc diffusion method, and a new categorization of antibiotics based on resistance levels was proposed. The results showed that *Staphylococcus aureus* isolates exhibited high resistance to various commonly prescribed antibiotics, with significant differences among antibiotic classes. Multidrug resistance showed temporal variations, with a peak of 54.29% in 2021. The study suggested that empirical treatment guidelines may need revision, and regional variations in antimicrobial resistance rates should be considered. These results also highlight the dynamic nature of antimicrobial resistance and indicate the need for continuous surveillance and rational use of antibiotics to prevent the dissemination of resistant strains of *Staphylococcus aureus* from veterinary sources.

Keywords: AMR surveillance; resistance patterns; multidrug resistance; public health.



INTRODUCTION

Antimicrobial resistance (AMR) is a serious global health challenge that can cause millions of deaths and enormous economic losses if not addressed urgently (MARSTON *et al.*, 2016; MEDINA *et al.*, 2020). AMR occurs when bacteria and other microorganisms develop the ability to withstand the effects of antibiotics and other antimicrobial agents, making infections harder to treat and increasing the risk of disease transmission (CHOKSHI *et al.*, 2019). One of the main drivers of AMR is the excessive and inappropriate use of antibiotics in human and veterinary medicine, which creates selective pressure for the emergence and spread of resistant bacteria (ADHIKARI *et al.*, 2023; CHOKSHI *et al.*, 2019). One of the primary strategies to combat AMR is to promote antimicrobial stewardship, which is the coordinated intervention to improve and measure the appropriate use of antimicrobials by promoting the selection of the optimal antimicrobial drug regimen, dose, duration, and monitoring (MARSTON *et al.*, 2016).

Antimicrobial stewardship is especially important in veterinary settings, where antibiotics are widely used for animals and can facilitate the transmission of resistant bacteria to humans through food or direct contact (FOOD & ADMINISTRATION, 2018). Several regulatory agencies such as the U.S. The Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have developed action plans and recommendations to monitor and control AMR in animals and animal products (AGENCY, 2019). However, in many countries, especially those with low or middle income, they have difficulties following these guidelines or lack adequate surveillance systems to monitor and control AMR in animals and animal products.

Among the AMR pathogens that can affect both humans and animals, *Staphylococcus aureus* (*S. aureus*) is a major pathogen that can cause a range of infections and food poisoning due to its toxin production (JFOSTER, 2002; J. ZHANG *et al.*, 2022). *S. aureus* is normally harmless when it colonizes the skin or mucous membranes, but it can become virulent when it invades deeper tissues or the



bloodstream through wounds or other breaches of the immune system (MICHAEL *et al.*, 2014; TONG *et al.*, 2015; TURNER *et al.*, 2019). Moreover, *S. aureus* can acquire resistance to multiple antibiotics, such as methicillin-resistant *S. aureus* (MRSA), which poses a greater threat to public health (DEURENBERG *et al.*, 2007).

S. aureus is a persistent and growing problem despite advances in molecular technologies and therapeutics (VAN BIJNEN *et al.*, 2015). Therefore, it is crucial to conduct regular and comprehensive surveillance of AMR from veterinary sources to inform evidence-based policies and practices. It is also important to understand the impact of antimicrobial usage in animals on the emergence and spread of AMR. In this paper, we investigate the AMR status of *S. aureus* isolated from animals and animal products in a veterinary hospital in Brazil. We discuss the challenges and opportunities for enhancing AMR surveillance, control, and hope that our findings will contribute to global efforts to combat AMR and protect human and animal health.

METHODOLOGY

Bacterial Isolates

This study collected 3,435 clinical specimens from a broad spectrum of domestic and wild animals between January 2017 and October 2023. All specimens originated exclusively from veterinary sources and included animals admitted for treatment at the Veterinary Hospital of the University of Passo Fundo (HV-UPF, Brazil), as well as samples submitted from external veterinary clinics and institutions for processing at HV-UPF's microbiology laboratory. Aseptic collection techniques were employed to minimize contamination during sample collection. All samples were inoculated onto mannitol salt agar (MSA) media (Himedia, India) and incubated at 37°C for 24 hours. Following incubation, presumptive *S. aureus* isolates were subcultured onto 5% sheep blood agar at 37°C for another 24 hours. A definitive identification of *S. aureus* was performed using a battery of standard biochemical tests. An isolate was confirmed as *S. aureus* if it displayed the following characteristics: acid production from mannitol on MSA media, beta-hemolysis of red blood cells on sheep blood agar, round, white/cream



colored colonies consistent with staphylococci, Gram-positive cocci in clusters, positive reaction (production of gas bubbles) for the catalase test, negative reaction (no color change) for the oxidase test, positive reaction (coagulation of rabbit plasma) for the coagulase test, positive reaction (conversion of nitrate to nitrite) for the nitrate reduction test, negative reaction (no ammonia production) for the urease test, negative reaction (no indole production) for the indole test, and non-motility in the motility test (LANCETTE *et al.*, 2020; SILVA *et al.*, 2000).

Antibiotic Susceptibility Evaluation

The antibiotic susceptibility of confirmed samples was assessed using the disc diffusion method by the conventional agar disk-diffusion procedure (Salam, Al-Amin, Pawar, Akhter, & Lucy, 2023). The antibiotics tested were gentamicin (10 µg), kanamycin (30 µg), neomycin (30 µg), tobramycin (10 µg), streptomycin (10 µg), cefaclor (30 µg), cephalexin (30 µg), cephalothin (30 µg), cefazolin (30 µg), ceftiofur (30 µg), cephalexin + neomycin, chloramphenicol (30 µg), florfenicol (30 µg), vancomycin (30 µg), lincomycin (2 µg), erythromycin (15 µg), azithromycin (15 µg), nitrofurantoin (300 µg), metronidazole (50 µg), amoxicillin (10 µg), ampicillin (10 µg), oxacillin (1 µg), penicillin G (10 µg), amoxicillin + clavulanic acid (30 µg), ciprofloxacin (5 µg), enrofloxacin (5 µg), levofloxacin (5 µg), norfloxacin (10 µg), ofloxacin (5 µg), sulfonamide (300 µg), trimethoprim (5 µg), sulfamethoxazole + trimethoprim (25 µg), doxycycline (30 µg), and tetracycline (30 µg). The zones of growth inhibition were measured and interpreted according to the CLSI standards for antimicrobial disk susceptibility tests for bacteria isolated from animals (VET01S) (CLINICAL & INSTITUTE, 2015). The criteria in CLSI M100-S24 were followed for antibiotics not covered by VET01S (Y. ZHANG AND WANG, 2014). *S. aureus* ATCC 25923 was used as the control strain.

The data obtained were evaluated for the presence of multidrug resistance (MDR) following the guidelines set by Sweeney, Lubbers, Schwarz, and Watts (2018). An isolate was considered MDR if it showed resistance to at least one antibiotic from three or more antibiotic classes. This study also proposed a new categorization of antibiotics based on



their resistance levels: highly resistant ($>60\%$ AMR), intermediate resistance (30 to 60% AMR), and low resistant ($<30\%$ AMR).

Statistical Analysis

A weighted least squares analysis and a chi-square test with Bonferroni correction were performed to examine the significance and the relationship of the antibiotic classes over the years, respectively. A p value below 0.05 was considered statistically significant (HERNANDEZ et al., 2021). The results of the chi-square test were expressed as odds ratios (ORs) with 95% confidence intervals (CIs). Both analyses were performed using GraphPad Prism software version 8.3.

Results and Discussion

A total of 3,435 specimens were analyzed at HV-UPF, of which 261 (7.6%) were confirmed *S. aureus* upon culture and biochemical tests. Subsequently, all the confirmed samples were submitted to the same antibiotic susceptibility evaluation using the disc diffusion method against a panel of thirty-four different antibiotics. This resulted in 2,144 individual susceptibility determinations.

AMR profile

The resistance levels of *S. aureus* strains were influenced by the type of antibiotics used (Figure 1). The antibiotics with the highest resistance levels ($> 60\%$) were metronidazole, penicillin G, sulfonamide, azithromycin, kanamycin, cephalexin + neomycin, erythromycin, and streptomycin. The antibiotics with moderate resistance levels (30-60%) were cephalothin, isonoxacin, amoxicillin, tobramycin, norfloxacin, sulfamethoxazole + trimethoprim, trimethoprim, ciprofloxacin, tetracycline, oxacillin, levofloxacin, cephalexin, and lincomycin. The antibiotics with the lowest resistance levels ($< 30\%$) were enrofloxacin, amoxicillin + clavulanic acid, ampicillin, gentamicin, ceftiofur, neomycin, doxycycline, cefazolin, cefaclor, florfenicol, vancomycin, chloramphenicol, and nitrofurantoin.

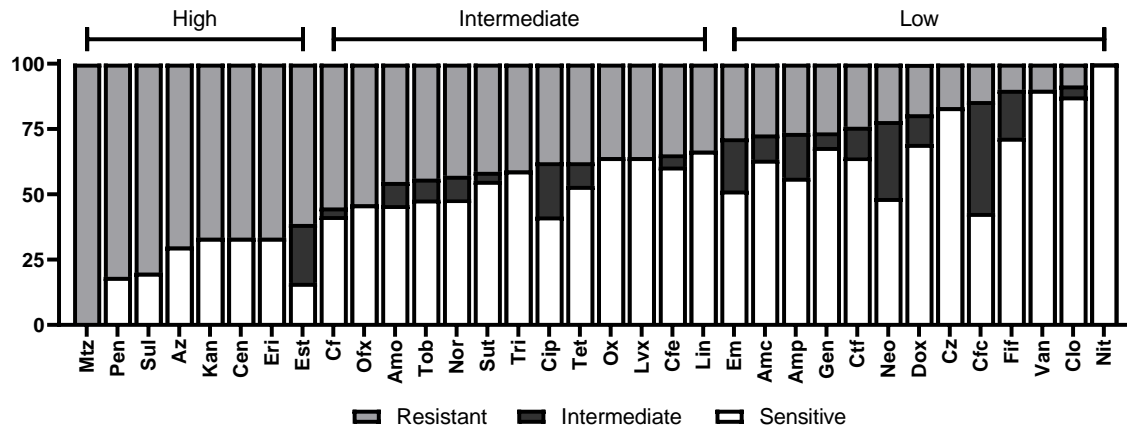


Figure 1. Susceptibility of *S. aureus* to different antibiotics from 2017 to 2023. The bars show the percentage of isolates that were resistant (gray), intermediate (black), or sensitive (white) to each antibiotic. The antibiotics are abbreviated as follows: Mtz (metronidazole), Pen (penicillin G), Sul (sulfonamide), Az (azithromycin), Kan (kanamycin), Cen (cephalexin + neomycin), Eri (erythromycin), Est (streptomycin), Cf (cephalothin), Ofx (isonoxacin), Amo (amoxicillin), Tob (tobramycin), Nor (norfloxacin), Sut (sulfamethoxazole + trimethoprim), Tri (trimethoprim), Cip (ciprofloxacin), Tet (tetracycline), Ox (oxacillin), Lvx (levofloxacin), Cfe (cephalexin), Lin (lincomycin), Em (enrofloxacin), Amc (amoxicillin + clavulanic acid), Amp (ampicillin), Gen (gentamicin), Ctf (ceftiofur), Neo (neomycin), Dox (doxycycline), Cz (cefazolin), Cfc (cefaclor), Fif (florfenicol), Van (vancomycin), Clo (chloramphenicol), and Nit (nitrofurantoin). The bars above antibiotics classify the antibiotics into highly resistant (>60% of AMR), intermediate resistant (30 to 60% of AMR), and low resistance (<30% of AMR).



The resistance levels also showed significant differences among the classes of antibiotics (Figure 2). The lincosamide class had the highest resistance level (83.33%), while the nitrofurantoin class had the lowest resistance level (0%). The chi-square test demonstrated that the resistance levels among the classes of antibiotics were significantly different ($p < 0.0001$), indicating that some classes of antibiotics were more effective than others against *S. aureus* strains.

These results revealed that antibiotics commonly used for empirical treatment, such as penicillin, lincosamides, sulfonamides, and macrolides, had high or intermediate resistance levels, ranging from 35.43% to 83.33% (DAVID AND DAUM, 2017). This implies that empirical treatment, which is a method of treating infections based on the presumed source of infection without waiting for laboratory tests, may not be effective for *S. aureus* infections. Therefore, it is suggested that empirical treatment guidelines should be updated to align with the current resistance patterns and to optimize treatment outcomes.

The results also indicated that the AMR rates were not consistent among different antibiotics within the same class, so selecting treatment by class may not be appropriate. This may be due to the different mechanisms of action of these antibiotics, even though they are in the same class. For instance, kanamycin and streptomycin are aminoglycosides that bind to the 30S ribosomal subunit and are deactivated by aminoglycoside-modifying enzymes (AMEs), which are widespread among *S. aureus* strains (X. ZHANG *et al.*, 2021). Gentamicin, however, is an aminoglycoside that binds to both the 30S and 50S ribosomal subunits and is less affected by AMEs (HODIAMONT *et al.*, 2022). Therefore, the observed trends are in line with the expected patterns, as gentamicin had a lower AMR rate than kanamycin and streptomycin.

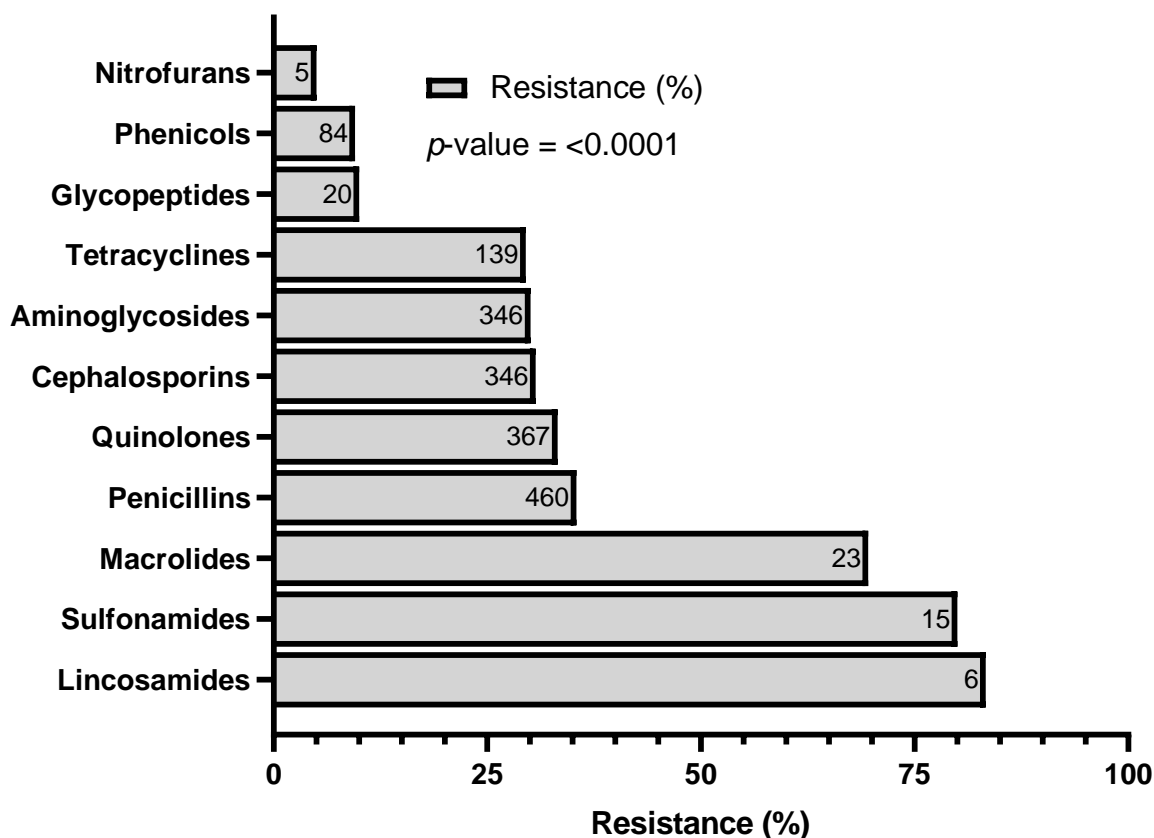


Figure 2. Resistance rates of *S. aureus* strains against different classes of antibiotics. The x-axis shows the percentage of resistance, while the y-axis shows the different classes of antibiotics. The p value is less than 0.0001, indicating a significant difference in the resistance patterns among the classes of antibiotics. The number inside each bar represents the sample size for each class of antibiotics.

A similar pattern was observed among penicillin. Penicillin G, which has been used for a longer period than ampicillin, showed a much higher AMR rate (81.63% to 26.44%) (CLARKE, 2015). This suggests that the long history of penicillin G use may have induced selective pressure on bacteria to develop resistance mechanisms.

Overall, these results have important implications for public health, as *S. aureus* infections are a major cause of morbidity and mortality, especially when they are resistant to antibiotics (ADHIKARI *et al.*, 2023). Therefore, it is essential to monitor the susceptibility patterns of bacteria to different antibiotics to prevent the emergence and



spread of AMR and to optimize clinical outcomes and reduce treatment costs (DAVID AND DAUM, 2017).

It is also essential to acknowledge regional variations in AMR rates. Tetracycline, for example, had an AMR rate in Ethiopia of 62% in 2016, which is higher than the 37.66% found in this study (DEYNO *et al.*, 2017). In contrast, the gentamicin resistance rate observed in this study was 26.26%, which is higher than the findings from a study in low- and middle-income countries, where the rate was less than 20% (VAN BOECKEL *et al.*, 2019).

MDR profile

The MDR of *S. aureus* exhibited temporal fluctuations from 2017 to 2023, as shown in Figure 3. The resistance percentage increased from 38.1% in 2017 to 54.29% in 2021, with a peak of 53.33% in 2020. A decline in resistance was observed in 2022, reaching 25%. However, the resistance percentage rebounded to 40% in 2023. When the data were analyzed by the linear regression model, a low R² value of 0.0039 was found, which indicated that it only explained a small proportion of the variation in the data.

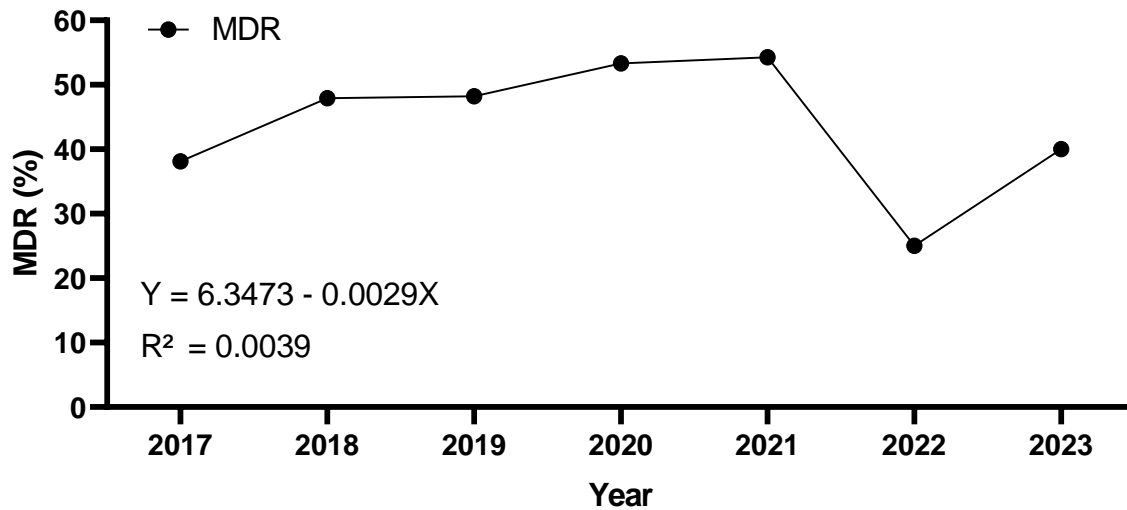


Figure 3. Multidrug Resistance (MDR) of *S. aureus* from 2017 to 2023. The x-axis represents the year, and the y-axis represents the resistance percentage. The equation for the line is $Y = 6.3473 - 0.0029X$.

Compared to other studies, it was possible to note that the MDR rate varies depending on the region, period, and bacterial species. For instance, a study in Nepal in 2022 found that 52% of isolates of *S. aureus* from a teaching hospital were MDR, while another study in European countries reported that only 7.1% of nasal commensal isolates had such resistance (Table 1) (ADHIKARI *et al.*, 2023; VAN BIJNEN *et al.*, 2015).

**Table 1.** Multidrug Resistance Rates of *S. aureus* by Country and Source (2004 – 2023).

Source	MDR ¹	Country	Reference
Veterinary Hospital	43.84%	Brazil	This study
Retail Meat	10.40%	United States	(GE <i>et al.</i> , 2017)
Meat and Poultry	52.00%	United States	(WATERS <i>et al.</i> , 2011)
Hog Slaughter	6.90%	United States	(NEYRA <i>et al.</i> , 2014)
Teaching Hospital	52.00%	Nepal	(ADHIKARI <i>et al.</i> , 2023)
Nasal Commensal	7.10%	Europe	(VAN BIJNEN <i>et al.</i> , 2015)
Milking Farms	34.30%	Kenya	(SHITANDI AND STERNESJO, 2004)
Companion Animals	44.68%	Italy	(SCARPELLINI <i>et al.</i> , 2023)

¹Multidrug Resistance. The MDR determination method may vary for each study.

The high rate of MDR found in this study presents a substantial public health concern due to the well-established correlation between MDR and increased virulence in *S. aureus* strains. Specifically, MDR *S. aureus* frequently presents upregulated virulence factors, resulting in more severe and recalcitrant infections (RASMI *et al.*, 2022). Furthermore, MDR *S. aureus* presents a zoonotic risk, with the potential for transmission between animals and humans, particularly in agricultural settings where frequent human-livestock contact occurs (CHOKSHI *et al.*, 2019).

Understanding the specific MDR profiles circulating within veterinary populations is crucial to mitigating the potential "spillover" of resistant strains into human populations (THAKUR AND GRAY, 2019). The alarmingly high rates of MDR observed in *S. aureus* isolated from other veterinary sources, as noted in Table 1, further emphasize the urgency of this issue. Therefore, vigilant monitoring and stringent



antimicrobial stewardship are essential to track the emergence and spread of MDR *S. aureus* strains in veterinary settings (FOOD & ADMINISTRATION, 2018).

These monitoring studies also align with global stewardship to combat the spread of AMR. The World Health Organization (WHO) maintains a global database of results of the Tripartite AMR Country Self-Assessment Survey (TrACSS) to provide information on the status of countries regarding the implementation of their national action plans and actions to address AMR across all sectors (EL OMEIRI *et al.*, 2023). According to the TrACSS report from 2023, Brazil is currently “jointly working on issues including agreement on common objectives”. However, the report also highlights that Brazil has not yet implemented AMR education or used scientific data, such as the data presented in this work, to inform operational decision-making and amend policies. We hope that with more information, such as the information presented in this work, Brazil’s policies can be improved.

CONCLUSIONS

This study revealed a high and complex resistance scenario of *S. aureus* to some commonly used antibiotics. The study found temporal variations in the MDR rate of *S. aureus*, suggesting that the MDR situation is not static but dynamic. By assessing the AMR and MDR of *S. aureus*, this study contributed to global efforts to tackle AMR as a major public health challenge. This highlights the importance of local surveillance and evidence-based treatment to mitigate the impact of AMR and MDR on health and the economy. Furthermore, the study emphasized the need for more research and innovation to develop new strategies and solutions to combat AMR and improve patient outcomes and global health security.

AUTHORSHIP CONTRIBUTION STATEMENT

RL and LBR: Conceptualization, Writing – review & editing. LRS and LBR: Funding acquisition. LP, MZB, TAA, and BWK: Investigation. RL: Methodology, Writing – original draft. FP, LRS, and LBR: Supervision.



DECLARATION OF COMPETING INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ACKNOWLEDGMENTS

The Brazilian National Council of Research (CNPq) supported this work.

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