





# Antimicrobial resistance and *mecA* gene in *Staphylococcus* spp. isolates from horses used in family farming

## Resistência Antimicrobiana e Gene *mecA* em *Staphylococcus* spp. Isolados de cavalos usados na agricultura familiar

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**ABSTRACT:** Horses can contribute to the spread of bacterial diseases, which can be caused mainly by, *Staphylococcus* spp., which are part of the animals' commensal microbiota, but it is also considered a pathogenic microorganism capable of causing serious infections. vancomycin, when it is resistant to methicillin. Antimicrobial resistance is considered a major health problem by the World Health Organization and the emergence of the *mecA* gene, responsible for resistance to the class of beta-lactam antibiotics. Thus, the aim of this work was to investigate the antimicrobial resistance profile and the presence of the *mecA* gene in *Staphylococcus* spp. isolated from the nasal, oral and auricular microbiota of horses used as animal traction on small family farms. Nasal, oral and auricular swabs were collected from 38 horses, with 29 (76.3%) isolated in nasal swab, 15 (39.4%) in auricular swab and 9 (23.6%) in oral swab, totaling 53 *Staphylococcus* spp. and 50 (94.33%) samples were resistant to the 11 antimicrobials tested, none of which were positive for molecular tests to identify the *mecA* gene. The results demonstrate the presence of *Staphylococcus* spp. associated with high (94.33%) bacterial resistance, indicating that horses can be considered reservoirs of multi-resistant microorganisms.

**KEYWORDS:** equine; *mecA* gene; microorganism; resistance; *Staphylococcus* spp.

**RESUMO:** Os equinos podem contribuir para a disseminação de doenças bacterianas, podendo ser causadas principalmente pelo, *Staphylococcus* spp., que fazem parte da microbiota comensal dos animais, mas também é considerado microrganismo patogênico capaz de causar infecções graves, em seu tratamento o medicamento mais utilizado é a vancomicina, quando há resistência a meticilina. A resistência antimicrobiana é considerada um dos principais problemas de saúde pela Organização Mundial de Saúde e o surgimento do gene *mecA*, responsável pela resistência à classe dos antibióticos beta-lactâmicos. Deste modo, o objetivo deste trabalho foi investigar o perfil de resistência antimicrobiana e a presença do gene *mecA* em *Staphylococcus* spp. isoladas da microbiota nasal, oral e auricular de cavalos usados como tração animal em pequenas propriedades familiares. Foram coletados swabs nasal, oral e auricular de 38 cavalos, sendo identificados 29 (76,3%) isolados em swab nasal, 15 (39,4%) em swab auricular e 9 (23,6%) em swab oral, totalizando 53 *Staphylococcus* spp. e 50 (94,33%) amostras foram resistentes aos 11 antimicrobianos testados, nenhuma amostra foi positiva aos testes moleculares para identificação do gene *mecA*. Os resultados demonstram a presença de *Staphylococcus* spp. associada à alta (94,33%) resistência bacteriana, indicando que os cavalos podem ser considerados reservatórios de microrganismos multirresistentes.

**PALAVRAS-CHAVE:** equino; gene *mecA*; microrganismo; resistência; *Staphylococcus* spp.

## INTRODUCTION

The relationship between man and horses has a long history and over time this animal species has become important in human life, serving as an essential tool for transportation, hunting, war,

and meat as a means of food (MILLS; NANKERVIS, 2005). With about 6 million horses (IBGE, 2020), horse agribusiness acts directly on the Brazilian economy, where the equine industry moved about R\$ 16.15 billion and the activity generates

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Received: 12/08/2021. Accepted: 06/21/2022

610 thousand direct jobs and 2,430 thousand indirect jobs, being responsible, therefore, for three million jobs (MAPA, 2016). Thus, knowing the health of these animals is essential in the context of efficiency and One Health.

The equine species, as well as other domestic animal species, can contribute to the spread of bacterial diseases of importance in One Health (WIDODO; EFFENDI; KHAIRULLAH, 2020) and in this context can be found *Staphylococcus* spp., bacteria belonging to the group of gram-positive cocci, which are part of the animals' commensal microbiota. However, it is also a pathogenic microorganism capable of causing serious infections such as abortions, stillbirths, infectious arthropathies, skin lesions, among many others (SANTANA, 2015; WIDODO; EFFENDI; KHAIRULLAH, 2020).

To treat these staphylococcal infections, it is necessary to choose the correct drug and vancomycin is the antibiotic of choice for isolates resistant to methicillin (GUARDABASSI et al., 2010), since antimicrobial resistance is currently considered by the World Health Organization (2020) to be one of the greatest world problems. This fact raises the alert for a possible lack of new antibiotics, which may favor the spread of resistant bacteria, since they require higher concentrations of antimicrobials than the therapeutic doses provide, providing long-term inefficiency of the drug's action as well as the worsening health of the affected animal (SALABERRY et al., 2016).

Another situation that draws attention is the appearance of the *mecA* gene, responsible for resistance to the beta-lactam antibiotic class. This gene encodes the modified penicillin-binding protein and the misuse of antimicrobials in the management of animals contributes to the transmission of these resistant microorganisms (PAHISSA, 2009; WIDODO; EFFENDI; KHAIRULLAH, 2020).

Due to the importance of the equine species for society and economy, linked to the importance of multidrug-resistant microorganisms, the objective of this work was to investigate the profile of antimicrobial resistance and the presence of the *mecA* gene in *Staphylococcus* spp. isolated from the nasal, oral and auricular microbiota of horses used as animal traction on small family farms.

## MATERIAL AND METHODS

This project was submitted to the Ethics Committee on Animal Experimentation (CEPEEA) of UNIPAR and was approved under protocol number 30306/2016 on 12/03/2015.

In the period from March to September 2016, swabs were collected from the nasal, oral, and auricular cavities of 38 crossbred horses, of both sexes, over two years of age and used as animal traction in small family farms in the city of Umuarama, northwestern region of the state of Paraná. This region is also considered a border region, as it is located 130

km from Paraguay. All horses within the selected properties had samples collected.

At the time of the collection of biological material, the horses did not show clinical signs of any disease. Samples from the nasal, oral, and auricular cavities were collected using sterile swabs containing AIMES + activated carbon (CopanTransystem®, Italy). A swab was introduced into the oral cavity of each horse and circular and rotational movements were performed in the gingival and tongue regions. Subsequently, a second nasal swab was collected, compressing it with rotational movements. Finally, using the same movements, samples were collected from the auricular region. All samples were kept under refrigeration and immediately sent to the Laboratory of Preventive Veterinary Medicine and Public Health of the Postgraduate Program in Animal Science with an Emphasis on Bioactive Products of the Universidade Paranaense (UNIPAR).

The swabs were introduced individually in tubes containing 3 mL of Brain Heart Infusion (BHI) medium and incubated in a greenhouse at 37 °C for 24 hours. After this period, the cultures obtained were streaked on plates containing Mannitol Salt Agar and incubated at 37 °C for 24 hours to isolate gram-positive aerobic bacteria. The predominant colonies on each plate were isolated and streaked in the BHI medium, incubated at 37 °C for 24 hours, and later stored in 10% glycerol at a temperature of - 20 °C (QUINN et al., 1994).

Each isolate was subjected to analysis of macroscopic characteristics, Gram staining, and biochemical tests (QUINN et al., 1994). Gram-positive catalase-positive cocci were subjected to coagulase testing for classification into coagulase-positive *Staphylococcus* or negative coagulase *Staphylococcus*.

The polymerase chain reaction (PCR) was performed for the samples in the coagulase-positive *Staphylococcus* (StCP) to verify which of these isolates were *Staphylococcus aureus*. The DNA was extracted with the PurelinkGenomic DNA Kit (Invitrogen, Carlsbad, California, USA) according to the manufacturer's information and the reactions were performed using the primer Sa442-1 (5'-AAT CTT TGT CGG TAC ACGATA TTC ACG-3') and the Sa442-2 (5'-CGT AAT GAGATT TCA GTA GAT AAT ACA ACA-3') following the methodology of Martineau et al. (1998). For the amplification of DNA, the AppliedBiosystems thermocycler, model Veriti™ 96-Well ThermalCycler, was used.

The amplification of the products was visualized by electrophoresis on a 2% agarose gel stained with GelRed (Uniscience, Osasco, São Paulo, BR) using a 100 bp molecular marker and the products were visualized as a single band of 241 bp.

Antimicrobial susceptibility tests were performed using the disk agar diffusion methodology according to the criteria established by the Clinical Laboratory and Standards Institute (QUINN et al., 1994). Isolated colonies were seeded in the BHI medium for overnight growth. On the day of the experiments, the bacterial inoculum was standardized according to

the 0.5 McFarland standard and the bacterial suspension was inoculated into plates containing Mueller-Hinton agar with the aid of a swab. Subsequently, disks impregnated with antimicrobials were placed and the plates incubated (37 °C/18-24 h). The inhibition halos were measured (mm) and the results obtained were recorded.

The following antimicrobials were tested for gram positive bacteria: Amoxicillin (10µg), Ampicillin (10µg), Ciprofloxacin (5µg), Enrofloxacin 05 (5µg), Gentamicin (10µg), Norfloxacin (10µg), Oxacillin (1µg), Penicillin (10µg), Sulfazotrim (25µg), Tetracycline (30µg), Vancomycin (30µg).

The DNA of *Staphylococcus* spp. isolates classified as resistant and with intermediate resistance to oxacillin were extracted using the PurelinkGenomic DNA Kit (Invitrogen, Carlsbad, California, USA), according to the manufacturer's information, and the PCR reactions were performed using Platinum Supermix (Invitrogen, Carlsbad, California, USA); primer *mecA1* (AAAATCGATGGTAAAGGTTGG) and *mecA2* (AGTTCTGCAGTACCGGATTTG) at 5 µM; the DNA of the isolates and nuclease-free water totaling 25 µL. For amplification, the thermocycler of the brand "Thermo", model Px2 ThermalCycler, was used, under the parameters described by Murakami et al. (1991).

The amplification of the products was visualized by electrophoresis in 2% agarose gel stained with GelRed (Uniscience, Osasco, São Paulo, BR), and the products were visualized as a single band of 533 bp.

To determine multidrug resistance, the MAR index was used, defined as **a/b**, where **a** will be the number of antimicrobials to which the isolates were resistant and **b** the number of antimicrobials to which the isolate was exposed. Values above 0.200 suggest samples of high risk to public health (KRUMPERMAN, 1983).

## RESULTS

Swabs were collected from 38 crossbred horses and from both sexes. Of the total samples analyzed, 29 isolates of *Staphylococcus* spp. were identified, 29 (76.3%) isolated from nasal swab, 15 (39.4%) from auricular swab, and 9 (23.6%) from oral swab, totaling a total of 53 *Staphylococcus* spp. isolated.

All 29 (100%) isolates of *Staphylococcus* spp. from nasal swabs were confirmed as coagulase-negative. Of the 15 auricular isolates, 10 (66.6%) were coagulase-negative and 5 (33.3%) coagulase positive. Of the 9 oral samples, 6 (66.6%) were coagulase-negative and 3 (33.3%) positive.

Of the 8 coagulase-positive *Staphylococcus* spp. samples, only 1 (12.5%) was identified as *Staphylococcus aureus*. Among the 53 samples, 50 (94.33%) were resistant to the 11 tested antimicrobials.

Regarding the disk diffusion test, 27 (93.1%) of the nasal samples showed resistance to Penicillin, 22 (75.8%) to Ampicillin, 20 (68.9%) to Oxacillin, 18 (62.0 %) for Vancomycin, 17 (58.6%) for Amoxicillin, 15 (51.7%) for Sulfazotrim, 12 (41.3%) for Enrofloxacin and Gentamicin, 11 (37.9%) for Tetracycline, 8 (27.5%) for Ciprofloxacin and 1 (3.4%) for Norfloxacin (Tab. 1).

In view of the 15 (100%) auricular samples, the greatest resistance was to Ampicillin with 11 (73.3%), followed by Penicillin and Sulfazotrim with 8 (53.3%), Oxacillin with 6 (40.0%), Amoxicillin and Tetracycline with 2 (13.3%) and Norfloxacin, Gentamicin, and Vancomycin with 1 each (6.6%) (Tab. 1)

When the 9 oral samples were analyzed, the resistance profile was 8 (88.8%) resistant to Sulfazotrim, 6 samples (66.6%) resistant to Oxacillin and Penicillin, 5 (55.5%) to Ampicillin, 3 (33.3%) to Tetracycline, and 1 (11.1%) to Vancomycin and Amoxicillin each (Tab. 1).

**Table 1.** Resistance profile, by antimicrobial, of *Staphylococcus* spp. isolates from 29 nasal samples, 15 auricular samples, and 9 oral samples from crossbred horses used as animal traction in small family farms in the city of Umuarama, northwest region state of Paraná, 2016

Antibiotic	Total Swabs		Nasal Swab		Ear Swab		Oral Swab	
	R	%	R	%	R	%	R	%
Amoxicillin	20	27.6%	17	58.6%	02	13.3%	01	11.1%
Ampicillin	38	68.2%	22	75.8%	11	73.3%	05	55.5%
Ciprofloxacin	08	9.1%	08	27.5%	00	0.0%	00	0.0%
Enrofloxacin	12	13.7%	12	41.3%	00	0.0%	00	0.0%
Gentamicin	13	15.9%	12	41.3%	01	6.6%	00	0.0%
Norfloxacin	02	3.3%	01	3.4%	01	6.6%	00	0.0%
Oxacillin	32	58.5%	20	68.9%	06	40.0%	06	66.6%
Penicillin	41	71.0%	27	93.1%	08	53.3%	06	66.6%
Sulfazotrim	31	64.6%	15	51.7%	08	53.3%	08	88.8%
Tetracycline	16	28.1%	11	37.9%	02	13.3%	03	33.3%
Vancomycin	20	26.5%	18	62.0%	01	6.6%	01	11.1%

Caption: R = Resistant; % = Percentage.

The MAR index of nasal samples ranged from 1, resistance to all antibiotics, to 0, sensitive to all antimicrobials. Ear samples ranged from 0.09 to 0.36 and oral samples from 0 to 0.45 (Tab. 2 and 3).

Regarding the *mecA* gene, no sample was positive in molecular tests.

## DISCUSSION

Equinoculture has become an increasingly productive and promising activity for Brazil. The equine market moved around R\$ 25 billion in 2019, placing this activity as one of the most important in agribusiness (MILLS; NANKERVIS, 2005). Horses are used in different activities and can be used for work, sport, transportation, riding, agriculture, by police, and even as a therapeutic and educational method through hippotherapy.

Despite the countless benefits that these animals offer, they can also expose to different risks to human health, caused by the transmission of pathogenic microorganisms (NORMANNO et al., 2007; PAHISSA, 2009; SALABERRY et al., 2016).

In this work, *Staphylococcus* spp. was researched and detected in nasal, auricular, and oral swabs from crossbred horses used as animal traction and these results corroborate the studies by Normanno et al. (2007) in Umuarama, Paraná, Pahissa (2009) in Bagé, Rio Grande do Sul and Salaberry et al. (2016) in Fortaleza, Ceará, who also detected this microorganism in the nasal, oral and auricular cavities of horses, respectively.

This bacterial species is considered as an opportunistic pathogen that produces a wide variety of extracellular

toxins and virulence factors, which are related to pathogenicity and the resistance mechanisms to the available antimicrobials. This shows its high importance for One Health given the possibility of transferring antimicrobial resistance genes between bacteria of animal and human origin, as well as between bacteria of the normal microbiota and pathogenic microorganisms of different origins (SALABERRY et al., 2016).

In this work, *Staphylococcus aureus* was detected in only one sample. Guardabassi et al. (2010) highlighted in the study with horses the same pathogen isolated and multi-drug-resistant to conventional antimicrobials. *Staphylococcus aureus* is a bacterium commonly found in the mucosa and conjunctiva of humans and animals, related to different clinical infections and the biggest problem of this agent is its resistance to different antibiotics, so infections caused by this microorganism is an emerging problem in horses with the

**Table 3.** Index of bacterial multiresistance of *Staphylococcus* spp. isolates from nasal, auricular and oral swabs from crossbred horses used as animal traction in small rural family farms in the city of Umuarama, northwest region of the state of Paraná, 2016

Bacterial Resistance Index		
Samples	R	%
N 29	163	0.510%
A 15	40	0.242%
O 09	29	0.292%

Caption: N: nasal, A: auricular; O: oral

**Table 2.** Index of bacterial multiresistance of nasal, auricular and oral isolates of *Staphylococcus* spp. from 29 crossbred horses used as animal traction in small family farms in the city of Umuarama, northwest region of the state of Paraná, 2016.

Nasal Samples				Ear samples		Oral Samples	
R	%	R	%	R	%	R	%
06	0.545%	02	0.181%	01	0.090%	00	0.0%
06	0.545%	01	0.090%	03	0.272%	04	0.363%
09	0.818%	02	0.181%	01	0.090%	03	0.272%
07	0.636%	09	0.818%	01	0.090%	04	0.363%
07	0.636%	05	0.454%	01	0.090%	05	0.454%
06	0.545%	08	0.727%	02	0.181%	02	0.181%
08	0.727%	10	0.909%	04	0.363%	02	0.181%
09	0.818%	06	0.545%	01	0.090%	04	0.363%
08	0.727%	08	0.727%	04	0.363%	05	0.454%
11	1.0%	06	0.545%	02	0.181%		
09	0.818%	01	0.090%	05	0.454%		
07	0.636%	00	0.0%	05	0.454%		
02	0.181%	02	0.181%	06	0.545%		
03	0.272%	03	0.272%	01	0.090%		
02	0.181%			03	0.272%		

Caption: Krumperman (1983) suggests that values above 0.200 indicate high-risk samples

possibility of transmission to workers who have contact with these animals (WANNMACHER, 2004; GUARDABASSI et al., 2010; SANTANA, 2015; WIDODO; EFFENDI; KHAIRULLAH, 2020).

Related to bacterial resistance, in this study it was found that penicillin was the most prevalent (71.0%) in all biological samples (nasal, auricular, and oral swabs), followed by ampicillin (68.2%) in nasal and auricular swabs and sulfazotrim (64.6%) in auricular and oral samples. A similar situation has also been described by Arias et al. (2012) in a university veterinary hospital in the state of Colorado (USA) between 1994 and 2001 where they detected that 54% of hospitalized horses received antimicrobials for the treatment of different conditions, with the use of penicillin and ampicillin being prescribed in 39% of cases, and more specifically, penicillin showed resistance in 93.1% of cases while ampicillin in 73.3%.

Pansani et al. (2016) verified more than 90% susceptibility to sulfazotrim in samples collected from nasal swabs from horses, not corroborating the results found in this research, since sulfazotrim demonstrated high bacterial resistance (51.7%) in the analyzed samples.

Although oxacillin showed significant results in terms of bacterial resistance in this study, the *mecA* gene was not detected, a more favorable situation, since this resistance gene has no action on beta-lactam class antimicrobials, the most used of which they are penicillin, ampicillin, amoxicillin, and oxacillin (SANTANA, 2015). The use of antimicrobials for therapeutic, prophylactic purposes, or as a growth stimulator (GUARDABASSI et al., 2010) in agriculture is already known and its indiscriminate use can stimulate the appearance of resistance in the bacterial population (NORMANNO et al., 2007; SALABERRY et al., 2016) and later cause damage to other animals, man and the environment itself.

Another situation that calls attention is that the MAR index indicated that in this study 64.10% of the samples were considered to be of high risk, with 61.70% of the nasal orifice, 20.50% of the ear, and 17.60% of the oral cavity. These results are worrisome when it comes to horses used as animal

traction in small family farms since the owners and/or collaborators of the properties are exposed to this situation. Santana (2015) reports in her work the genetic similarity and patterns of bacterial resistance of *Staphylococcus* in equines in different Brazilian regions, indicating the diversity of isolates throughout the national territory, suggesting that these animals may be involved in the transmission of pathogenic strains to other animals, humans, and even the environment, which raises some concern when it comes to One Health due to the difficulty of controlling this pathogen and the absence of new drugs available to combat it.

## CONCLUSION

The results of this work demonstrate the presence of *Staphylococcus* spp. associated with high (94.33%) bacterial resistance in horses used for animal traction of small rural properties of family farming and among these, only one isolate was identified as *Staphylococcus aureus*. Future molecular studies must be conducted to identify the possible species involved in these horses.

These results indicate that horses can be considered as reservoirs of multidrug-resistant microorganisms, being a serious problem for One Health, considering the possibility of dissemination among bacteria of the equine microbiota and the owners/workers involved. In addition, the importance of carrying out susceptibility tests to different antimicrobials is evident for the knowledge of the ideal drug for the institution of treatments in horses, thus avoiding the possibility of increasing resistance and stimulating the presence of the *mecA* resistance gene.

## ACKNOWLEDGMENTS

To the owners, for their constant collaboration; to UNIPAR and to the Fundação Araucária (CP 09/2016 Programa Institucional de Pesquisa Básica e Aplicada - Protocolo: 47661.491.48325.12122016 e CP 10/2018 Programa Pró-Equipamentos - Protocolo: 51176.543.48325.29102018) for the financing granted to this research; to CAPES for granting the PROSUP school fee and to CNPq CP 12/2017 - Research Productivity Grants.

## REFERENCES

ARIAS, M. V. B.; CARRILHO, C. M. D. M. Resistência antimicrobiana nos animais e nos humanos. Há motivo de preocupação? **Semina: Ciências Agrárias**, Londrina, v. 33, n. 2, p. 775-790, 2012.

BRASIL. MINISTÉRIO DA AGRICULTURA, PECUÁRIA E ABASTECIMENTO. **Revisão do Estudo do Complexo do Agronegócio do Cavalo**. Brasília, 2016. 54 p.

GUARDABASSI, L.; JENSEN, L. B.; KRUSE, H. **Guia de antimicrobianos em veterinária**. 1. Ed. Porto Alegre: Artmed, 2010. 268 p.

IBGE - INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. **PPM - Produção da Pecuária Municipal 2020**. Rio de Janeiro, 2020. Disponível em: <https://www.ibge.gov.br/estatisticas/economicas/agricultura-e-pecuaria/9107-producao-da-pecuaria-municipal.html?=&t=destaques>

KRUMPERMAN, P. H. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. **Applied and Environmental Microbiology**, v. 46, p. 165-170, 1983.

- MARTINEAU, F. et al. Species-specific and ubiquitous-DNA-based assays for rapid identification of *Staphylococcus aureus*. **Journal of Clinical Microbiology**, v. 36, p. 618-623, 1998.
- MILLS, D.; NANKERVIS, K. **Comportamento Equino: Princípios e Prática**. 1. Ed. São Paulo: Roca, 2005. 213 p.
- MURAKAMI, K. et al. Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. **Journal of Clinical Microbiology**, v. 29, p. 2240-2244, 1991.
- NORMANNO, G. et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) in foods of animal origin product in Italy. **International Journal of Food Microbiology**, Amsterdam, v. 117, n. 2, p. 219-222, 2007.
- PAHISSA, A. **Infecciones producidas por *Staphylococcus aureus***, 1. Ed. Barcelona: ICG Marge, SL, 2009. 183 p.
- PANSANI, A. M. et al. Prevalência e resistência a antibióticos de (*Streptococcus equi*) da cavidade nasal de equinos hígidos no município de Fernandópolis, São Paulo, Brasil. **Acta Veterinaria Brasilica**, v. 10, n. 2, p. 144-149, 2016.
- QUINN P.J. et al. **Clinical Veterinary Microbiology**. 1. Ed. London: Wolfe publishing, 1994. 648 p.
- SALABERRY, S. R. S. et al. Análise microbiológica e perfil de sensibilidade do *Staphylococcus* spp. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, Belo Horizonte, v. 68, n. 2, p. 336-344, 2016.
- SANTANAA, F. **Cultura e sensibilidade bacteriana da conjuntiva ocular de equinos de Brasília, Brasil**. 2015. 22 p. Trabalho de conclusão de curso (Graduação em Medicina Veterinária) – Universidade de Brasília/Faculdade de Agronomia e Medicina Veterinária, 2015.
- WANNMACHER, L. Uso indiscriminado de antibióticos e resistência microbiana: Uma guerra perdida? **Uso racional de medicamentos: temas selecionados**, Brasília, v. 1, n. 4, p. 2-3, 2004.
- WIDODO, A.; EFFENDI, M. H.; KHAIRULLAH, A. R. Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* from livestock. **Systematic Reviews in Pharmacy**, v. 11, n. 7, p. 382-392, 2020.
- WORLD HEALTH ORGANIZATION. **Clinical Management of COVID-19: Interim Guidance**. Geneva: World Health Organization, 2020. 65 p.

