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[*Comunicação*]

Multidrug resistance associated with *Staphylococcus spp*. in canine ocular diseases

[*Multirresistência associada a* Staphylococcus *spp. em doenças oculares de cães*]

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Different ocular diseases can involve bacterial infections in dogs (Hewitt *et al*., 2020), due to the presence of normal ocular microbiota, which can lead to infection depending on various factors related to the breakdown of anatomical barriers, patient immunity, virulence of the etiological agent and presence of microorganisms (Whitley and Hamor, 2021)

The ocular microbiota can vary according to location, sampling method, season and previous treatments (Whitley and Hamor, 2021). Despite differences, there is a consensus that Gram-positive bacteria outnumber Gram-negative bacteria, with the genus Staphylococcus being the main representative of Gram-positive bacteria, while *Pseudomonas aeruginosa* is observed as the core representative of Gram-negative bacteria (Hewitt *et al*., 2020).

In cases of bacterial infection, the use of antimicrobial agents is necessary for resolving the condition or delaying lesion progression (Hewitt *et al*., 2020). It is essential to identify the microbial agent present and its susceptibility to available antimicrobial agents in ophthalmic formulations (Hindley *et al*., 2016). Inappropriate or ineffective prescription may not only fail to resolve the condition but also contribute to the development of antimicrobial resistance (Goss *et al*., 2023).

Bacterial resistance occurs on a global scale in humans, companion animals and production animals (McEwen and Collignon, 2018). It is established by both pathogenic and non-pathogenic bacteria, posing a One health challenge. Therefore, monitoring resistance profiles is crucial as it serves as an epidemiological and therapeutic indicator in the control of ocular infections and helps prevent various risk situations such as infection by multidrug-resistant bacteria in humans (Soimala *et al*., 2018). Several factors contribute to bacterial resistance among ocular pathogens, including short and repeated exposure to antimicrobials, which are identified as contributors to ocular pathogen resistance and alteration of ocular microbiota (Goss *et al*., 2023).

In a study where samples from dogs and cats with various ocular diseases like conjunctivitis, blepharitis, keratitis and uveitis were collected, methicillin resistance was traced in *Staphylococcus spp.* isolates. It was observed that they also exhibited resistance to β-lactams due to the presence of resistance genes, as well as to erythromycin, clindamycin, streptomycin, gentamicin, enrofloxacin, tetracycline, and sulfamethoxazole-trimethoprim (Soimala *et al*., 2020).

It is also possible to observe a growing increase in multidrug resistance associated with ophthalmic conditions, as in the study of Hewitt *et al.* (2020), who described that multidrug-resistant (MDR) isolates accounted for 20% of all corneal isolates, with a incidence increasing of 5% in 2016 to 34% in 2020. Consequently, the development of strains with multidrug-resistant characteristics has become increasingly common in both human and veterinary medicine (McEwen and Collignon, 2018).

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Thus, the objective of this study was to evaluate bacterial samples obtained through conjunctival swabs from various ocular diseases in dogs between the years 2016 and 2021, aiming to observe the prevalence and evaluate the resistance of the isolated bacterial strains.

MATERIAL AND METHODS

The samples were collected using swabs and sterile transport media from dogs treated in the Veterinary Ophthalmology Service at the Veterinary Hospital, Faculty of Veterinary Medicine, Unesp, Araçatuba Campus, São Paulo, between the years 2016 and 2021. The samples were immediately sent to the Veterinary Microbiology laboratory after collection. Culture, identification and antimicrobial susceptibility tests were performed by disk diffusion according to Clinical and Laboratory Standards Institute (2023) guidelines.

A descriptive analysis of 49 samples was conducted. The antimicrobials evaluated (Table 1.) were chosen by the attending veterinarian responsible for the case and varied accordingly. All reports included patient identification, species, age, occurrence and bacterial identification.

Table 1 Classification of the selected antimicrobials for the disk diffusion test by pharmacological class and sub-class

Class	Sub-class	Antimicrobial agent
Aminoglycosides	-	Amikacin Gentamicin Neomicyn Tobramycin
Cephalosporins	1st gen	Cefalexin
	3rd gen	Ceftiofur Ceftriaxone
	4th gen	Cefepime
Carbapenems	-	Imipenem
Penicillins	-	Amoxicillin Amoxicillin/clavulanic acid Ampicillin Oxacillin Penicillin
Chloramphenicol	-	Chloramphenicol
Lincosamides	-	Clindamycin
Fluoroquinolones	2nd gen	Ciprofloxacin Enrofloxacin Norfloxacin Ofloxacin
	3rd gen	Marbofloxacin Moxifloxacin
Sulfonamides	-	Sulfadiazine/Trimethoprim
Tetracyclines	-	Doxycycline Tetracycline
Polymyxins	-	Polymyxin B
Nitroimidazoles	-	Metronidazole
Macrolide	-	Azithromycin Erythromycin Spiramycin

The results were classified as sensitive, resistant or intermediate, based on the specific resistance displayed by each identified pathogen to each tested antimicrobial. The presence of multidrug resistance was attributed to pathogens exhibiting resistance to at least one drug belonging to three or more different classes of antimicrobials, or when the strain showed resistance to a carbapenem (Magiorakos *et al.*, 2012).

RESULTS

Of the evaluated dogs (n=49), 61.22% (30/49) were males and 38.77% (19/49) were females. Among these, brachycephalic dogs accounted for 69.38% (34/59), with shih-tzu breed being the majority among the individuals (58.82%) (20/34).

Samples were collected from different ocular diseases, with ulcerative keratitis being the most frequent in this retrospective study, accounting for 86.67% (41/49) of all cases (Fig. 1).

Bacterial growth was identified in 61.22% (30/49) of the samples, with 20.00% (6/30) of these showing a co-infection, characterized by the growth of more than one bacterial species. In 38.77% (19/49) of cases, there was no growth of any bacterial species.

Among the isolated species (Fig. 2), Gram-positive bacteria were the most found, accounting for 80.56% (29/36) of all isolates, with *Staphylococcus spp*. being the primary representative with 66.67% (24/36), followed by species of *Streptococcus spp*. with 13.89% (5/36) and *Bacillus* spp 2.78% (1/36). Gram-negative bacteria accounted for 16,67% (6/36) of the isolates, with representatives including *Proteus spp*. at 8.33% (3/36), Pseudomonas aeruginosa with 5.56% (2/36), and *Escherichia coli* with 2.78% (01/36).

Overall, the samples considered MDR comprised 18.36% (9/30), exhibiting resistance primarily to antimicrobial agents commonly used in veterinary ophthalmology clinical practice such as ciprofloxacin 77.78% (7/9), tobramycin 55.56% (5/9), chloramphenicol 44.44% (4/9), and ofloxacin 33.33% (3/9). Among all samples considered multidrug-resistant, the genus *Staphylococcus* accounted for 77.78% (7/9) of the MDR samples.

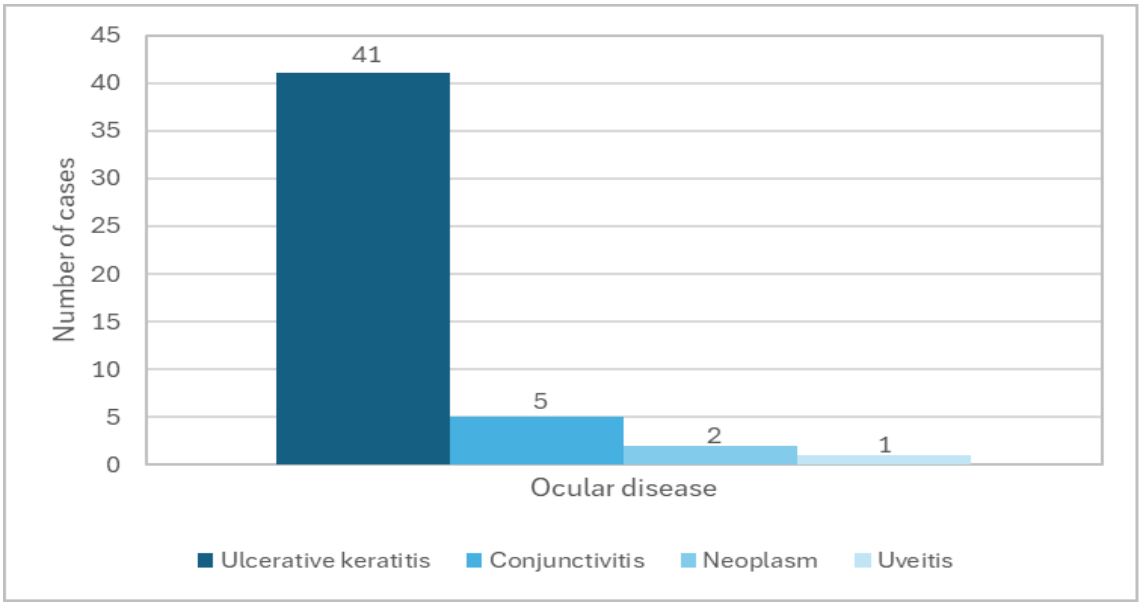

Figure 1. Distribution of ocular diseases attended at the Veterinary Ophthalmology Service of the Veterinary Hospital "Luiz Quintiliano de Oliveira" at the Faculty of Veterinary Medicine of Araçatuba - Unesp, Araçatuba Campus, São Paulo, between the years 2016 and 2021, from which samples from the ocular surface were collected for culture, isolation and antibiogram.

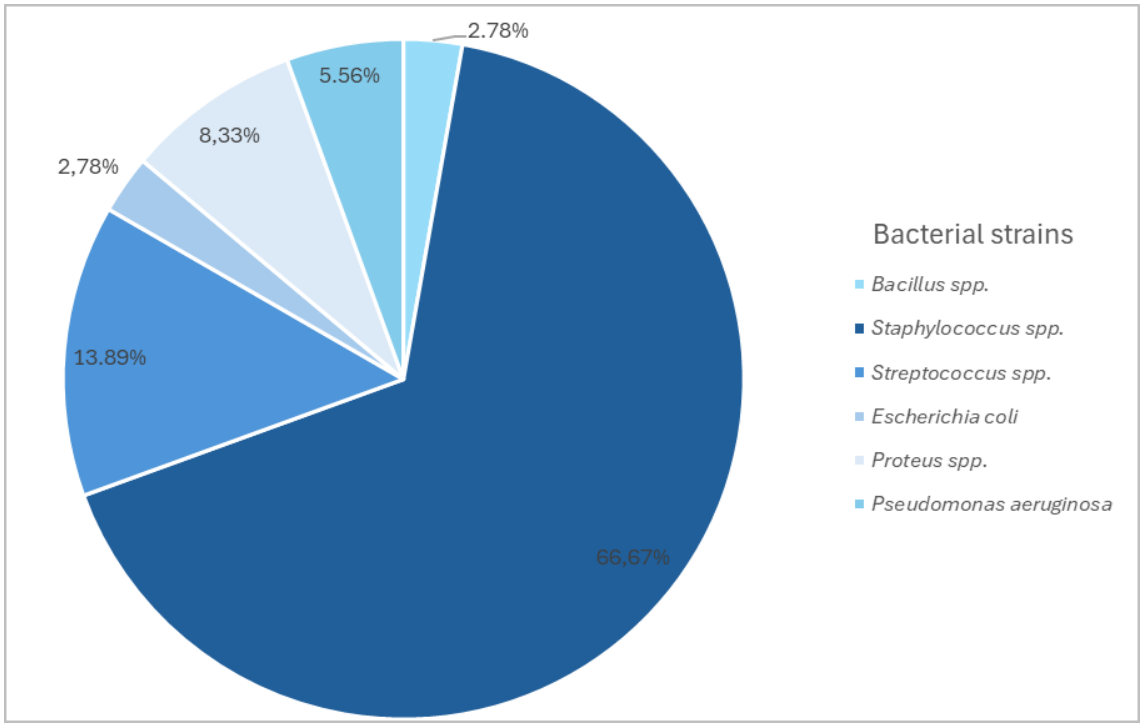

Figure 2. Bacterial strains isolated from ocular diseases attended at the Veterinary Ophthalmology Service of the Veterinary Hospital "Luiz Quintiliano de Oliveira" at the School of Veterinary Medicine of Araçatuba - Unesp, Araçatuba Campus, São Paulo, between the years 2016 and 2021.

DISCUSSION

Ulcerative keratitis comprised most ophthalmic occurrences in this study, with a significant portion of the evaluated dog population being brachycephalic breeds, particularly shih-tzus. When associating breed with corneal disease, it is widely accepted that corneal ulcers stem from various etiologies (Goss *et al*., 2023)., with such causes often linked to brachycephalic animals due to their facial conformational alterations, predisposing them to brachycephalic ocular syndrome and the onset of this condition (Hindley *et al*., 2016).

Ulcerative keratitis, along with any eyelid or conjunctival lesions, is typically contaminated by commensal bacteria from the ocular microbiota. Hence, antimicrobial use is necessary in most cases for recovery or to halt the progression of ophthalmic clinical conditions (Verdenius *et al*., 2023). Antimicrobial prescription is usually empirical due to the rapid advancement of these ocular diseases; however, it is essential and fundamental to perform bacterial identification and antimicrobial susceptibility tests for more accurate treatment (Goss *et al*., 2023).

In this study, the most frequently isolated bacterium was *Staphylococcus spp*., which is expected as this genus is part of the normal conjunctival microbiota in healthy animals (Soimala *et al*., 2020), or those presenting some form of ocular disease (Hindley *et al*., 2016). Studies have observed different bacterial genera, such as *Streptococcus sp*. and *Pseudomonas aeruginosa*, indicating that the infection profile may involve a wide bacterial diversity (Verdenius *et al*., 2023). This highlights the importance of identifying the causative agent for the treatment of any ocular condition, especially those related to the ocular surface.

Staphylococcus spp. is commonly associated with cases of antimicrobial resistance, both in human and veterinary health, thus representing an important aspect for one health. It is known that the genus possesses various mechanisms of resistance to antimicrobials that may be administered (Kadlec *et al*., 2015). Consequently, the indiscriminate use of these drugs increases

the chance of developing multidrug-resistant conditions. In veterinary ophthalmology, a considerable risk factor for the development of MDR strains is the administration of antibiotic eye drops before referral to specialized care. Thus, this promotes the iatrogenic selection of resistant bacteria due to their repeated use (Goss *et al*., 2023).

Multidrug resistance associated with *Staphylococcus* spp. species can be considered high (Kadlec *et al*., 2015). For instance, we can observe that infections resistant to *Staphylococcus* spp. pose a serious challenge in the healthcare of both human and veterinary patients (Goss *et al*., 2023). Therefore, the presence of resistant *Staphylococcus* strains raises concern, as animals can share human microbiota due to the proximity in which they live (Jung *et al*., 2020), therefore promoting the transmission of resistant strains between humans and dogs (Goss *et al*., 2023).

Given that this is a retrospective clinical study, the absence of some data hindered a thorough evaluation, as it was not possible to collect the data uniformly. Only antimicrobials common to all animals were included. The number of eyes evaluated in this study reflects the reality that ocular conditions are often neglected when screening for resistant microorganisms. The choice of antimicrobials evaluated is also a limiting factor of the study, as it was the responsibility of the attending veterinarian and varied for each patient. The variation made challenging to assess resistance over the years, serving instead as a clinical indicator of the presence of resistant agents. However, understanding the epidemiology of infectious agents and their resistance profiles in these ocular clinical cases provides valuable information. This knowledge promotes greater awareness of effective therapeutic approaches for each clinical condition.

CONCLUSION

This study concludes that the most frequently isolated genus in ocular conditions in dogs in the city of Araçatuba is *Staphylococcus* spp. However, other species were also isolated over the years, demonstrating a diverse ocular microbiota capable of causing infection. We observed that this bacterial genus is also associated with multidrug resistance. Emphasizing the importance of early identification and monitoring resistance patterns is crucial for effective treatment and reducing risk of bacterial resistance.

Keywords: antibiotics, bacteria, dogs, one health

RESUMO

Doenças oculares em cães são suscetíveis à infecção bacteriana. O tratamento dessas condições envolve o uso de antimicrobianos, portanto a identificação e o teste de suscetibilidade do agente causador devem ser realizados para promover a recuperação clínica e evitar o desenvolvimento de resistência bacteriana. Dessa maneira, o presente estudo teve como objetivo documentar os isolados de doenças oculares entre os anos de 2016 e 2021, na cidade de Araçatuba-SP, onde a ceratite ulcerativa foi a condição mais observada, representando 86,67% (41/49) dos casos. O crescimento bacteriano foi identificado em 61,22% (30/49) de todas as amostras, em que 20% (6/30) dessas apresentaram coinfecção, enquanto em 38,77% (19/49) dos casos não houve crescimento de nenhuma espécie bacteriana. Entre as espécies isoladas, as bactérias Gram-positivas representaram 80,56% (29/36), sendo mais comumente encontrado Staphylococcus *spp. como principal representante, compreendendo 66,67% (24/36) dos isolados.As bactérias Gram-negativas representaram 16,67% (6/36). Entre as amostras, as consideradas multirresistentes compreendiam 18,36% (9/30), sendo principalmente resistentes a agentes antimicrobianos como ciprofloxacina em 77,78% (7/9), tobramicina em 55,56% (5/9), cloranfenicol em 44,44% (4/9) e ofloxacina em 33,33% (3/9). De todas as amostras consideradas multirresistentes, o gênero Staphylococcus representou 77,78% (7/9) das amostras multirresistentes.*

Palavras-chave: antimicrobianos, bactéria, cães, saúde única

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