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Conjunctival bacterial flora and antimicrobial susceptibility of captive and free-living sea turtles in Brazil

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Abstract

Purpose: To describe the aerobic conjunctival bacterial flora of 3 especies of free-living and under human care sea turtles and determine its antimicrobial susceptibility in vitro.

Method: Thirty-six sea turtles (72 eyes), juveniles and adults, 7 free-living *Chelonia mydas* and 8 *Chelonia mydas*, 4 *Caretta caretta*, 11 *Eretmochelys imbricata*, and 6 *Lepidochelys olivacea* under human care, were evaluated. Conjunctival cultures were collected for identification of aerobic bacteria and antimicrobial susceptibility testing for ciprofloxacin, chloramphenicol, gentamicin, neomycin, oxacillin, polymyxin B, tetracycline, and tobramycin using antibiotic disks. Bacterial strains showing no sensitivity to 4 or more antimicrobials were considered multiresistant to this panel.

Results: Bacterial growth was observed in 12/14 (85.71%) samples in the free-living sea turtles, and there was growth in 100% (58/58) of the samples from captive animals. There were 94 strains isolated and 15 species identified. There was a predominance of Gram-positive bacteria in free-living *Chelonia mydas*, most of which were *Bacillus* and *Staphylococcus*. The most commonly isolated Gram-negative species were enterobacteria for free-living and under human care animals. The strains were predominantly sensitive to ciprofloxacin and tobramycin, and less sensitive to oxacillin or polymyxin B. Ten multiresistant strains were isolated. Yeast were identified in 13.89% (10/72) of the samples.

Conclusions: These results, showing differences in the conjunctival bacterial flora of free-living and captive animals, may be helpful for diagnosis and treatment of ocular disorders in sea turtles.

KEYWORDS
aerobic culture, *Caretta caretta*, *Chelonia mydas*, *Eretmochelys imbricata*, *Lepidochelys olivacea*, multidrug resistant

1 | INTRODUCTION

The sea turtles are classified into 6 genera with 7 species disseminated across all oceans. Five species are found on the Brazilian coast, and they all classified as threatened or endangered, mainly due to anthropogenic activity.[1-6] Phenotypic, behavioral, and nutritional features distinguish these species, such as the herbivorous diet of adult green sea turtles (*Chelonia mydas*),[7] the presence of imbricate scales on the hawksbill sea turtle (*Eretmochelys imbricata*), the olive-green shell color of the olive ridley sea turtle (*Lepidochelys olivacea*), and the large head of the loggerhead sea turtle (*Caretta caretta*).[8]

Ophthalmic features are quite similar among sea turtles and other chelonians—high corneal sensitivity,[9] the presence of scleral ossicles,[10,11] thick, strong eyelids,[12] small eyes relative to body size, and mucoid lacrimal secretions.[11] Vision is the most important sense in sea turtles, and ocular diseases can change their social and feeding behaviors, as well as their ability to escape from harm. Loss of vision may contribute to an increase in morbidity or mortality in sea turtle.[10,13,14]

The conjunctiva is in direct contact with the environment. Numerous microorganisms may reside in this mucosa, and these can change upon interaction with the habitat.[15-17] One of the main ophthalmopathies in sea turtles is fibropapillomatosis, caused by the α-herpesvirus subfamily.[18] The second most common is trauma.[18,19] Both conditions can result in secondary changes in the conjunctival flora or opportunistic infection by bacteria.[14]

The establishment of species-specific parameters, as conjunctival bacterial flora, may aid in the identification of pathogenic bacteria and may guide in the choice of antibiotic treatments. This study describes and evaluates the mesophilic and aerobic conjunctival bacterial flora in free-living and captive sea turtles from Brazil, and its antimicrobial susceptibility in vitro.

2 | MATERIALS AND METHODS

2.1 | Ethical considerations

The study was approved by the Biodiversity Authorization and Information System, Brazilian Ministry of the Environment (50054-2), and the Animal Ethics Committee of the Federal University of Bahia (50/2016). It was conducted in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research.

2.2 | Free-living sea turtles

Fourteen conjunctival swab samples were collected from 7 free-living *Chelonia mydas*. The animals were temporarily taken from the marine coast (lat. 12.5694 S, long. 37.9887 W) in a nylon net. Before collection, technical staff (TAMAR Project), and veterinary ophthalmologist (UFBA) identified and evaluated physical and ophthalmic conditions. Animals with indications of systemic disease or gross abnormalities of the eye or periocular region were excluded. The free-living *Chelonia mydas* were maintained out of water for 20-30 minutes and after sample collection were taken back to the same place. In collection period of free-living sea turtle, the temperature of water surface in marine coast was around 25-30°C (National Institute of Meteorology).

2.3 | Sea turtles under human care

Twenty-nine sea turtles (8 *Chelonia mydas,* 4 *Caretta caretta*, 11 *Eretmochelys imbricata,* and 6 *Lepidochelys olivacea*), born and kept in different visitor centers (VC) of the TAMAR Project (Brazil), were used in the study. Before collection, technical staff (TAMAR Project) and a veterinary ophthalmologist (UFBA) identified and evaluated physical and ophthalmic conditions. Animals with indications of systemic disease or gross abnormalities of the eye or periocular region were excluded. Fifty-eight conjunctival swab samples were obtained, and the total handling time out of water was 15-20 minutes per animal.

Identification and description of free-living and captive sea turtles are presented in Table 1. Some of the information on sex and age was not confirmed due to phenotypic features (absence of dimorphism), in some animals, and was thus classified as "undetermined".

2.4 | Food and habitat conditions for sea turtles under human care

The visitor center tanks follow the Standard permit conditions for care and maintenance of captive sea turtles.[20] Water of VC is taken from sea and filtered using sand filter. Once the water captured for use in the tanks being of marine origin, salinity is not regulated, only evaluated, which there was more variation in VC3, due to the proximity of the catchment site with riverbed (Table 2). The pH of water tanks was around 7.5-8.5, and chlorine was used during the washing of tanks for disinfection, and added to the water to reduce organic matter. The water from the tanks was then returned to the sea free of chlorine.

Diet of sea turtles under human care is based on fish inside the tanks, with the leftovers removed after feeding and tanks are vacuumed daily for stool and other material removal. Animals also received seaweed or vegetables and vitamin supplementation twice a week.

2.5 | Collect, culture of conjunctival flora with antimicrobial susceptibility test

All animals were only manually contained and after their removal from the water, a 10-minutes interval was performed for drainage of residual water from the ocular area.[21]

Samples were collected between autumn and winter (05/05/2017 to 13/07/2017), from the ventral conjunctival fornix of both eyes using sterile cotton swabs with rotating

TABLE 1 Identification and description of the species, sex, age, locality, curved carapace length (CCL), curved carapace width (CCW), and weight of the studied sea turtles

ID	Specie	Location	Gender	Age (years)	CCL (cm)	CCW (cm)	Weight (kg)
1	*Chelonia mydas*	Free-living	U	U	44	40	10.5
2	*C. mydas*	Free-living	U	U	75	84	>50
3	*C. mydas*	Free-living	U	U	76	72	>50
4	*C. mydas*	Free-living	U	U	56	51	33
5	*C. mydas*	Free-living	U	U	40	35	10
6	*C. mydas*	Free-living	U	U	69	64	>50
7	*C. mydas*	Free-living	U	U	74	73	>50
8	*C. mydas*	VC1	U	6	70	61	46.4
9	*C. mydas*	VC1	U	U	39	35	9
10	*C. mydas*	VC2	U	U	50	41	13.3
11	*C. mydas*	VC2	U	U	54	44	16.4
12	*C. mydas*	VC2	M	12	79	65	54
13	*C. mydas*	VC2	F	U	92	84	127
14	*C. mydas*	VC3	U	U	40	37	10
15	*C. mydas*	VC4	M	U	89	68	78
16	*Caretta caretta*	VC1	U	5	66	74	41.2
17	*C. caretta*	VC1	U	5	63	74	39.2
18	*C. caretta*	VC1	U	5	65	72	40.8
19	*C. caretta*	VC1	U	5	62	70	36.2
20	*Eretmochelys imbricata*	VC1	U	3	47	41	11.4
21	*E. imbricata*	VC1	U	6	66	57	32
22	*E. imbricata*	VC1	U	6	68	76	52.8
23	*E. imbricata*	VC1	U	U	69	57	36.4
24	*E. imbricata*	VC1	M	16	84	68	68
25	*E. imbricata*	VC1	U	3	56	43	11.7
26	*E. imbricata*	VC1	U	3	48	42	11.3
27	*E. imbricata*	VC1	U	3	48	41	11.7
28	*E. imbricata*	VC1	U	3	47	42	11.4
29	*E. imbricata*	VC1	U	3	46	42	11.6
30	*E. imbricata*	VC4	U	U	69	57	36.4
31	*Lepidochelys olivacea*	VC1	M	U	65	65	34
32	*L. olivacea*	VC1	M	15	68	64	38
33	*L. olivacea*	VC3	F	U	58	57	32
34	*L. olivacea*	VC3	F	U	58	60	37
35	*L. olivacea*	VC3	U	U	55	56	21
36	*L. olivacea*	VC4	F	U	68	68	46.8

F, female; M, male; U, undetermined; VC, visitor center (numbers 1-4 refer to different visitor's centers).

movements. The manipulator gently trailed the lower eyelid while inserting the swab, to avoid contact with eyelids and skin. Swabs were immediately placed in tryptose agar medium and sent to the Bacterioses Laboratory of the veterinary hospital at Federal University of Bahia. Culture of the microorganisms was performed using Petri dishes with 6% sheep blood agar, eosin methylene blue agar, and tryptose broth. Dishes were incubated at 37°C in an aerobic environment for 24-48 hours for identification of the mesophilic bacteria (growth range between 25-40°C).[22-24] After growth, the colonies were identified based on the presence or absence of hemolysis on blood agar, and morphological

TABLE 2 Water parameters of visitor centers

	Salinity (ppm)	Temperature (°C)	Water renovation system	Tanks water renovation	Chlorine
VC1	30	28	Open	100% daily and wash every 1-2 times a week	Added 0.5 ppm daily
VC2	30	22-25	Closed	Every 3 months	Added in stored water before arriving in tanks
VC3	16-30	26	Semi-open	5%-10% daily and total every 30 days	Added 0.5 ppm daily
VC4	30	28	Semi-open	10% daily and wash every 2-3 times a week	Not added

VC, visitor center (numbers 1-4 refer to different visitor center).

and biochemical characteristics according to routine laboratory techniques (catalase test, oxidation-fermentation test, coagulase test, oxidase test, methyl red test, motility test, triple sugar iron, indole production, Simmons citrate agar, urease, malonate, and carbohydrate fermentation: glucose, sucrose, lactose, mannitol, adonitol, arabinose, and dulcitol).[25] The smears were then stained according to the Rosenfeld technique for identification of yeast.

Antimicrobial susceptibility test was performed using the disk diffusion method recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines for veterinary isolates.[26] Each culture inoculum was seeded (subcultured on) in Mueller-Hinton agar and antimicrobial disks (ciprofloxacin, chloramphenicol, gentamicin, neomycin, oxacillin, polymyxin B, tetracycline, and tobramycin) were added at previously marked, equidistant positions. The dishes were incubated at 37°C for 24 hours, and analysis of zone inhibition size was performed. Bacterial isolates resistant to at least 4 antimicrobials were designated multiresistant isolates for this panel. Because the disk susceptibility testing has not been reproducible or standardized to interpret results for bacterial like *Bacillus* species therefore has being excluded from processing for susceptibility.[26]

3 | RESULTS

There was bacterial growth in 97.22% (70/72) of the samples: 12/14 (85.71%) samples in free-living sea turtles and 100% (58/58) of the samples from visitor center animals. In total, 94 isolates were obtained and 15 species were identified (Tables 3 and 4). Thirteen isolates and 4 species were identified in free-living *Chelonia mydas*, 27 isolates and 9 species in *C. mydas*, 8 isolates and 5 species in *Caretta caretta*, 28 isolates and 9 species in *Eretmochelys imbricata,* and 18 isolates and 8 species in *Lepidochelys olivacea* from visitor centers.

Isolated strains of free-living *Chelonia mydas* had predominance of Gram-positive bacteria (76.92%). Of the bacterial isolates in animals under human care, 34.57% were Gram-positive and 65.43% Gram-negative. The growth of Gram-positive and Gram-negative bacteria, respectively, for sea turtles in visitor centers was of 40.74% vs 59.26% for *Chelonia mydas*, 25% vs 75% for *Caretta caretta*, 28.57% vs 71.43% for *Eretmochelys imbricata,* and 38.89% vs 61.11% for *Lepidochelys olivacea*. There were differences in bacterial growth among VC as shown in Table 5.

The bacteria isolated in free-living and from visitor centers sea turtles were most susceptible to ciprofloxacin and tobramycin and least susceptible to oxacillin and polymyxin B. The bacterial susceptibility for free-living *Chelonia mydas* isolates is described in Table 6. The lowest antibiotic susceptibility was identified for *Micrococcus* and Staphylococcus species in *Lepidochelys olivacea* and *Escherichia coli* in *Caretta caretta* and *Eretmochelys imbricata* (Table 7).

Ten multiresistant strains were obtained in this study. Only 1 isolate of *Staphylococcus* species obtained from free-living animal, and 9 strains of sea turtles under human

TABLE 3 Identification and frequency of aerobic bacterial isolates from conjunctival of free-living *Chelonia mydas*

	No. of isolates	Total of isolates (%)
Gram-positive		
Bacillus species	1	7.69
Staphylococcus species	6	46.15
Staphylococcus epidermidis	3	23.08
Total	10	76.92
Gram-negative		
Enterobacter hafniae	3	23.08
Total	3	23.08
Total of isolates	13	100

TABLE 4 Identification and frequency of aerobic bacterial isolates from the conjunctiva of sea turtles under human care

	No. of isolates	Total of isolates(%)	*Chelonia mydas*	*Caretta caretta*	*Eretmochelys imbricata*	*Lepidochelys olivacea*
Gram-positive						
Bacillus species	19	23.46	9	1	7	2
Staphylococcus species	5	6.17	1	1	0	3
Staphylococcus epidermidis	2	2.47	1	0	1	0
Micrococcus species	1	1.23	0	0	0	1
Staphylococcus aureus	1	1.23	0	0	0	1
Total	28	34.57	11	2	8	7
Gram-negative						
Enterobacter hafniae	11	13.58	4	0	3	4
Klebsiella species	11	13.58	3	1	7	0
Escherichia coli	8	9.88	5	2	1	0
Pseudomonas species	7	8.64	2	0	3	2
Serratia species	5	6.17	1	0	0	4
Shigella species	4	4.94	1	0	3	0
Proteus species	3	3.70	0	3	0	0
Enterobacter aerogenes	2	2.47	0	0	2	0
Proteus mirabilis	1	1.23	0	0	1	0
Providencia stuartii	1	1.23	0	0	0	1
Total	53	65.43	16	6	20	11
Total of isolates	81	100	27	8	28	18

care: 2 *Staphylococcus* species (2 isolated in *Lepidochelys olivacea* from VC3), 3 *Escherichia coli* (2 isolated in *Caretta caretta* from VC1 and 1 isolated in *Eretmochelys imbricata* from VC1), 1 *Enterobacter aerogenes* (*Eretmochelys imbricata* from VC1), 1 *Micrococcus* species (*Lepidochelys olivacea* from VC3), 1 *Proteus* species (*Caretta caretta* from VC1) and 1 *Pseudomonas* species (*Eretmochelys imbricata* from VC1), with lower susceptibility to the antibiotics oxacillin, polymyxin B, tetracycline, neomycin, and gentamycin.

Yeast were identified in 10 of 72 samples (13.89%), with 1 growing in free-living *Chelonia mydas*, 1 in *Lepidochelys olivacea*, 3 in captive *Chelonia mydas* (2 fungi were identified as *Malassezia* species, from the phenotype presented), and 5 in *Eretmochelys imbricata*.

4 | DISCUSSION

The identification and evaluation of comparative conjunctival flora in free-living vs visitor center's sea turtles can contribute to the development of disease prevention, examination interpretation, and disease treatment protocols of captive or rehabilitated turtles in specific facilities.[27-29] Comparisons of free-living animals with those living in visitor centers reinforce the importance of species-specific studies and environmental influence on the microflora that may impact turtles held in managed systems.[30]

Normal conjunctival flora has been described in chelonians such as turtles[23,24] and tortoises.[23,28,29] Previous studies in sea turtles have reported conjunctival bacterial flora in sick animals.[2,14] The mostly bacterial isolated of chelonians and other ectothermic animals are classified as mesophilic and its optimal temperature for growth is 25-40°C,[23-25,28,29,31] similar to the present study. However, there are no such studies on healthy sea turtles in free life or under human care nor any description of the antimicrobial susceptibility of their microflora.

Most conjunctival culture samples in this study showed bacterial growth, as reported for *Chelonoides carbonaria*[29] and *Trachemys scripta elegans*.[24] The greatest number of isolates was found in *Chelonia mydas* and *Eretmochelys imbricata*, both from visitor center. Variation in number of isolates from the same species living in 2 different habitats has already been reported in penguins[30] and can be attributed to numerous water quality factors. This is highlighted by the lower bacterial species variety in free-living sea turtles.

There were more Gram-positive than Gram-negative bacteria in free-living C*helonia mydas*, similar to the green

TABLE 5 Bacterial isolated of captive sea turtles from different visitor centers

	No. of isolates	VC1 (n = 36)	VC2 (n = 8)	VC3 (n = 8)	VC4 (n = 6)
Gram-positive					
Bacillus species	19	10	2	3	4
Staphylococcus species	5	2	0	3	0
Staphylococcus epidermidis	2	2	0	0	0
Micrococcus species	1	0	0	1	0
Staphylococcus aureus	1	0	0	1	0
Total	28	14	2	8	4
Gram-negative					
Enterobacter hafniae	11	3	3	3	2
Klebsiella species	11	8	2	0	1
Escherichia coli	8	3	3	0	2
Pseudomonas species	7	3	1	3	0
Serratia species	5	3	0	0	2
Shigella species	4	4	0	0	0
Proteus species	3	3	0	0	0
Enterobacter aerogenes	2	2	0	0	0
Proteus mirabilis	1	1	0	0	0
Providencia stuartii	1	1	0	0	0
Total	53	31	9	6	7
Total of isolated	81	45	11	14	11

n, number of samples; VC, visitor center (numbers 1-4 refer to different visitor center).

TABLE 6 Antimicrobial disk susceptibility of aerobic bacterial isolates from the conjunctiva of free-living *Chelonia mydas*

	No. of isolates	CIP (%)	CLO (%)	GEN (%)	NEO (%)	OXA (%)	POL (%)	TET (%)	TOB (%)
Staphylococcus species	6	100	100	100	100	100	66.66	33.33	100
Staphylococcus epidermidis	3	83.33	100	83.33	100	66.66	83.33	50	83.33
Enterobacter hafniae	3	100	100	100	66.66	66.66	33.33	66.66	100

CIP, ciprofloxacin; CLO, chloramphenicol; GEN, gentamicin; NEO, neomycin; OXA, oxacillin; POL, polymyxin B; TET, tetracycline; TOB, tobramycin.

iguana,[22,32] tortoise,[29] and broad-snouted caiman.[33] The captive animals showed a higher incidence of Gram-negative bacteria. However, percentages of Gram-positive and Gram-negative bacteria in these animals did not diverge widely and were similar to the study of captive *Trachemys scripta elegans*.[24] The results found among different visitor centers may be associated with water quality parameters and/or animal density.

The high occurrence of Gram-negative bacteria found in sea turtles from visitor centers may be attributed to tear deficiences,[34] population density,[30] water quality parameters, and presence of food residues that favor the proliferation of enteric bacteria in the habitat.[23] Animals living in tanks eliminate their waste fecal material into an enclosed aquatic environment concentrating the numbers and exposure to coliforms. Previous studies have described the presence of Gram-negative and Gram-positive bacteria in reptilian ocular infections, emphasizing the importance of establishing the conjunctival bacterial flora, and a good water quality that can impact animal health.[23,35-37]

Bacillus and *Staphylococcus* species were the most commonly isolated Gram-positive bacteria, similar to previous studies in chelonians.[23,24,29] Among *Bacillus* species isolates, 95% (19/20) were found in animals from visitor centers, and most of the *Staphylococcus* species strains grew from free-living sea turtles (56.25%; 9/16). These microorganisms have been previously described in blepharoconjunctivitis (*Bacillus* species), panophthalmitis (*Staphylococcus* species),[12] and keratoconjunctivitis in sea turtles.[2]

TABLE 7 Antimicrobial disk susceptibility of aerobic bacterial isolates from the conjunctiva of sea turtles under human care

	No. of isolates	CIP (%)	CLO (%)	GEN (%)	NEO (%)	OXA (%)	POL (%)	TET (%)	TOB (%)
Chelonia mydas									
Staphylococcus species	1	100	100	100	100	0	0	100	100
Staphylococcus epidermidis	1	100	100	100	100	100	100	100	100
Shigella species	1	100	100	100	100	0	100	100	100
Enterobacter hafniae	4	100	75	100	75	0	50	50	100
Klebsiella species	3	100	100	100	100	0	66.66	66.66	100
Serratia species	1	100	100	100	100	0	100	100	100
Pseudomonas species	2	100	0	100	100	0	100	50	100
Escherichia coli	5	100	100	100	100	0	100	80	100
Caretta caretta									
Staphylococcus species	1	100	100	100	0	0	0	100	100
Proteus species	3	100	100	66.66	33.33	0	33.33	100	100
Escherichia coli	2	100	50	50	50	0	0	0	100
Klebsiella species	1	100	100	100	100	0	100	100	100
Eretmochelys imbricata									
Staphylococcus epidermidis	1	100	100	100	100	100	100	100	100
Enterobacter aerogenes	2	100	100	100	50	0	50	100	50
Klebsiella species	7	100	100	100	87.71	0	71.43	100	100
Escherichia coli	1	100	100	100	0	0	0	0	100
Proteus mirabilis	1	100	100	100	100	0	100	100	100
Shigella species	3	66.66	100	100	33.33	0	66.66	100	100
Pseudomonas species	3	100	33.33	100	66.66	0	33.33	33.33	100
Enterobacter hafniae	3	100	100	100	33.33	0	100	100	100
Lepidochelys olivacea									
Staphylococcus species	3	33.33	66.66	33.33	66.66	33.33	33.33	33.33	100
Staphylococcus aureus	1	100	100	100	100	0	100	100	100
Micrococcus species	1	100	0	0	100	0	0	100	0
Serratia species	4	100	100	100	100	0	100	75	100
Providencia stuartii	1	100	100	100	100	0	100	100	100
Enterobacter hafniae	4	100	100	100	100	25	75	100	100
Pseudomonas species	2	100	50	100	100	0	100	50	100

CIP, ciprofloxacin; CLO, chloramphenicol; GEN, gentamicin; NEO, neomycin; OXA, oxacillin; POL, polymyxin B; TET, tetracycline; TOB, tobramycin.

Enteric bacteria comprised 92.45% (49/53) of the Gram-negative bacteria in the conjunctival flora of sea turtles, with the highest percentages of *Enterobacter hafniae*, *Klebsiella* species, and *Escherichia coli*. The lower occurrence of these bacteria in free-living sea turtles may be due to habitat dilution and decreased fecal exposure.[30] These agents are present in the conjunctival flora of other species[22,29,32,33,38] and are described as potential pathogens in reptiles.[12,35-37] Differences in bacterial recovery isolation of individuals kept in tanks at different locations and facilities were noted, although the animals are subjected to similar food handling (feeding in an aquatic environment) and habitat handling (tanks with salty water). Factors such as density of animals per tanks may be associated with these differences.

Two isolates of the genus *Pseudomonas* have been reported in the normal conjunctival flora of turtles and tortoises,[23] similar to this study with captive *Chelonia mydas*, *Eretmochelys imbricata* and *Lepidochelys olivacea*. This genus has been described as an agent of conjunctivitis, blepharitis, exophthalmos, eyelid edema, uveitis, and abscesses in reptiles, maintained under conditions that may result in immunosuppression.[12,36,37]

Other microorganisms represented by only a few isolates, such as *Proteus* species in *Caretta caretta*, *Enterobacter aerogenes* in *Eretmochelys imbricata,* and *Providencia stuartii* in *Lepidochelys olivacea*, were observed and may suggest different environmental flora at the visitor center sites.

Antibiotic susceptibility reports of conjunctival flora in free-living animals are scarce. Ciprofloxacin and tobramycin are routinely used antibiotics in veterinary ophthalmology;[22,38,39] moreover, these antibiotics are used to treat sick animals by technical staff (TAMAR Project). The microorganisms isolated in the present study showed high sensitivity in 95.96% and 94.59% of the antibiograms performed for tobramycin and ciprofloxacin with these drugs suggesting these as first choice drugs until culture and sensitivity is available.

Gentamicin gave consistent susceptibility results for the isolated microorganisms, mainly the Gram-negative bacteria, similar to previous studies.[22,38-41] Treatment inefficacy,[12] as well as possible bacterial resistance mediated by plasmids has been documented.[42] It should be noted that aminoglycosides, such as gentamicin, neomycin, and tobramycin, have lower efficacy at temperatures below 30°C,[40] and it is recommended that the ambient temperature be raised when treating diseases in reptiles with this drug group.[43]

The isolates were moderately susceptible to chloramphenicol, neomycin, and tetracycline, presenting possible treatment alternatives in conjunctival infections. However, multiresistant strains showed less susceptibility to tetracycline and neomycin, and the conjunctival microbiota of free-living animals showed less sensitivity to tetracycline.

Most Gram-positive and negative bacteria isolated from animals under human care showed less susceptibility to oxacillin as well as to polymyxin B, except for *Staphylococcus epidermidis* isolates for oxacilin. The occurrence of bacterial strains with low sensitivity to antibiotics may restrict the choice of drugs for the treatment of eye infections.

In the present study, we identified multiresistant strains of *Staphylococcus* species, *Enterobacter aerogenes*, *Escherichia coli, Micrococcus* species, *Proteus* species, and *Pseudomonas* species, similar to Liu et al[44] who reported the antibiotic resistance of strains isolated in *Trachemys scripta elegans*. *Pseudomonas*[27,35,44] and *Escherichia coli*[45] have been reported as multidrug-resistant in other chelonians, allowing healthy animals to be disseminators of multidrug-resistant bacteria to other environments and hosts.[45]

The contact of some species of reptiles with humans motivated studies on the microbiota that these animals harbor and their antibiotic resistance.[23,24,28,29,44] The indiscriminate use of antimicrobials and its inappropriate discard in the aquatic environment, as well as natural resistance[27,31] may increase the occurrence of multiresistant bacteria, which have been reported in birds, reptiles, and mammals from aquatic environments.[2,27,46]

The fungal findings in this study do not present any immediate concern, as mycotic diseases are generally considered secondary to other infections, or to predisposing factors such as inadequate management. Nevertheless, they may cause skin, carapace, and eye lesions, often associated with infections.[12,47]

5 | CONCLUSION

The conjunctival flora of sea turtles showed a predominance of Gram-positive bacteria in free-living sea turtles and Gram-negative bacteria in different species of sea turtles maintained in 4 visitor centers. Most commonly isolated were *Bacillus* species, *Staphylococcus* species, and enterobacteria. Highest antibiotic susceptibility was to ciprofloxacin and tobramycin in all evaluated species, and lowest susceptibility was found to oxacillin and polymyxin B. Isolates from free-living animals were less sensitive to tetracycline, but in both, free-living and captive sea turtles, multiresistant bacteria were observed. These results show the influence of the environmental factors in the conjunctival cultures of visitor center's animals and free-living sea turtles and may be helpful in the diagnosis and treatment approach of ocular disorders in sea turtles.

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