

ORAL MICROBIOTA OF BRAZILIAN CAPTIVE SNAKES

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ABSTRACT: The present work aimed to determine the oral microbiotic composition of snakes from São José do Rio Preto city, São Paulo State, Brazil. Ten snake species, comprising the families Boidae, Colubridae, Elapidae and Viperidae, were submitted to microbiological examination of their oral cavity, which indicated positivity for all buccal samples. Gram-negative bacilli, gram-negative cocci bacilli, gram-positive bacilli and gram-positive cocci were isolated from the snakes. Among isolated bacterium species, the occurrence of coagulase-negative staphylococci in the buccal cavity of *Crotalus durissus* (Viperidae), *Eunectes murinus* (Boidae), *Mastigodryas bifossatus* (Colubridae) and *Bacillus subtilis*, common to oral cavity of *Bothrops alternatus* (Viperidae) and *Phalotris mertensi* (Colubridae), was detected. It was observed higher diversity of isolated bacteria from the oral cavity of *Micrurus frontalis* (Elapidae) and *Philodryas nattereri* (Colubridae), as well as the prevalence of gram-positive bacillus and gram-positive cocci. The composition of the oral microbiota of the studied snakes, with or without inoculating fangs, is diverse and also related to the formation of abscesses at the bite site in the victims of the ophidian accidents, and to pathogenic processes in the snakes that host these microorganisms.

KEY WORDS: venomous snakes, non-venomous snakes, oral cavity, bacterial isolation, captive snakes.

CONFLICTS OF INTEREST: There is no conflict.

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INTRODUCTION

Studies on oral microbiota of snakes are rare in Brazil and the existing reports are mainly on the *Bothrops* genus, which is involved in most of the reported accidents (1-5). Envenomation caused by this snake genus is characterized by inflammation and tissue destruction that leads to infection and abscess formation (6-9).

Among snakes that contribute to accidents in Brazil, four genera – *Micrurus* (with proteroglyph dentition); *Bothrops*, *Crotalus* and *Lachesis* (10, 6) (with solenoglyph dentition) – are considered common. However, some snakes with opisthoglyph and aglyph dentition, considered non-venomous, can also cause injuries at the bite site and, consequently, complications that determine the seriousness of the accidents (11).

Currently, several hypotheses try to explain infectious origins in lesions caused by ophidian accidents. In one of these theories, bacteria – from the venom, mouth and fangs of snakes – are involved (8, 11-14). Related research has been showing a strong relationship between microorganisms present in abscesses or in patient lesions (1, 2) and the ones from snake oral cavities (10, 11, 13).

In the present work, oral microbiota composition of snakes from the municipality of São José do Rio Preto, state of São Paulo, Brazil, was determined.

MATERIALS AND METHODS

All the analyzed snakes were from the municipality of São José do Rio Preto, state of São Paulo, and were maintained in captivity at the Animal House of São Paulo State University, São José do Rio Preto campus.

Animals that represented each one of ten snake species were chosen for oral cavity analysis. The ten species were: *Boa constrictor*, *Bothrops alternatus*, *Bothrops pauloensis*, *Crotalus durissus*, *Eunectes murinus*, *Mastigodryas bifossatus*, *Micrurus frontalis*, *Phalotris mertensi*, *Philodryas nattereri* and *Waglerophis merremii*.

The collection of buccal samples (teeth and mucous membrane) was performed after the animal had been immobilized. Its oral cavity was exposed and a swab, previously moistened in TSB (tryptic soy broth), was introduced. The samples were placed onto plates with blood agar (Difco, USA) and incubated at 36°C, for 24 hours, in the Microbiology Laboratory of São José do Rio Preto Medical School, FAMERP.

Gram staining was performed on all different types of colonies that had grown on the agar surface. Afterwards, each colony was isolated in brain heart infusion (BHI) agar

(Difco, USA) for subsequent biochemical identification using oxidase, catalase, sugar fermentation and DNase test.

Only one collected sample from each snake was used for the microbiological study. Bacteria isolated from snakes, classified according to genus and species, were analyzed by means of qualitative parameters for each buccal sample.

RESULTS

The test was positive for all buccal samples from animals that represented the following families: Boidae, Colubridae, Elapidae and Viperidae. Gram-negative bacilli (GNB), gram-positive bacilli (GPB), gram-positive cocci (GPC) and gram-negative cocci bacilli (GNCB) were isolated from each oral cavity. Higher bacterial diversity was found in the oral cavities of *M. frontalis* and *P. nattereri* (Table 1).

Bacterial classification, according to genus and species, was possible for *Actinomyces* sp. (*M. frontalis*), *Bulkolderia* sp. (*E. murinus*), *Moraxella* sp. (*M. frontalis*), *Proteus* sp. (*B. constrictor*), *Sarcina* sp. (*P. nattereri*), *Bacillus subtilis* (common in *B. alternatus* and *P. mertensi*), *Staphylococcus aureus* (*M. frontalis*), coagulase-negative staphylococci (*C. durissus*, *E. murinus*, *M. bifossatus*), and *Yersinia enterocolitica* (*B. pauloensis*).

Table 1. Microbiota isolated from oral cavities of studied snakes

Snakes	Family	Dentition	Microbiota
<i>Boa constrictor</i> Linnaeus, 1758	Boidae	Aglyph	GNB, GPB
<i>Eunectes murinus</i> Linnaeus, 1758	Boidae	Aglyph	GPC, GNGB
<i>Mastigodryas bifossatus</i> Raddi, 1820	Colubridae	Aglyph	GPC
<i>Phalotris mertensi</i> Hoge, 1955	Colubridae	Opistoglyph	GPB
<i>Philodryas nattereri</i> Steindachner, 1870	Colubridae	Opistoglyph	GPB, GPC, GNGB
<i>Waglerophis merremii</i> Wagler, 1824	Colubridae	Aglyph	GPB
<i>Micrurus frontalis</i> Duméril, Bibron & Duméril, 1854	Elapidae	Proteroglyph	GPB, GPC, GNGB
<i>Bothrops alternatus</i> Duméril, Bibron & Duméril, 1854	Viperidae	Solenoglyph	GPB
<i>Bothrops pauloensis</i> Amaral, 1923	Viperidae	Solenoglyph	GNB
<i>Crotalus durissus</i> Linnaeus, 1758	Viperidae	Solenoglyph	GPC

GNB: gram-negative bacilli; GPB: gram-positive bacilli; GPC: gram-positive cocci; GNGB: gram-negative cocci bacilli.

DISCUSSION

Goldstein *et al.* (15), analyzing the oral cavity of the North American rattlesnake, isolated the coagulase-negative staphylococci, *Proteus* sp. and *Pseudomonas* sp. In another study, on nonpoisonous snakes, they found the coagulase-negative staphylococci, *Acinetobacter* sp., *Hafnia alvei*, *Arizona hinshawii*, *Salmonella* sp., *Shigella* sp., *Klebsiella oxytoca* and *Pseudomonas aeruginosa* (16).

In the current study, concerning the Viperidae snakes, *Bacillus subtilis* was isolated from *B. alternatus*, *Yersinia enterocolitica* from *B. pauloensis* and a coagulase-negative staphylococcus from *C. durissus*.

Jorge *et al.* (3), studying the microbiota from fangs and venom of *Bothrops jararaca* in São Paulo State, most frequently isolated the group D streptococci, *Enterobacter* sp., followed by *Providencia rettgeri*, *Providencia* sp., *Escherichia coli*, *Morganella morganii* and *Clostridium* sp.; and less frequently *Staphylococcus aureus* and gram-positive bacilli.

In several Brazilian states, lesions caused by *Bothrops* envenomation – that were infected with gram-negative bacteria, gram-positive diplococcus, gram-positive cocci,

Clostridium sp., *Proteus* sp., *Enterobacter* sp. and *Klebsiella* sp. – have been reported. Analysis of these lesions demonstrated the presence of aerobic and anaerobic bacteria (3, 7, 8). Similarly, some of those bacteria found in lesions provoked by *Bothrops* were also isolated from the mouths of snakes studied in the present work, including *Proteus* sp. (from *Boa constrictor*), gram-positive bacilli and gram-negative bacilli from (*Philodryas nattereri*), coagulase-negative staphylococcus (from *Crotalus durissus*, *Eunectes murinus* and *Mastigodryas bifossatus*) and *Staphylococcus aureus* (from *Micrurus frontalis*).

Snakes can also host bacteria in their oral cavity. *Aeromonas* sp., *Enterobacter* sp., *Proteus* sp., *Pseudomonas* sp., *Staphylococcus* sp. and *Salmonella* sp. have already been isolated from wounds and abscesses of snakes; the same microorganisms were found in human abscesses (17-21).

Draper et al. (7), studying the microbiota from oral cavities of healthy and sick snakes, found gram-positive bacteria in healthy animals, whereas the sick ones presented, most frequently, *Pseudomonas*, *Providencia* and gram-negative bacilli.

In the current study, gram-positive bacilli were common to all oral cavities of the following snake families: Boidae, Colubridae, Elapidae and Viperidae.

These results reinforce what has been described in the literature (3, 12-14, 16, 22, 23), regarding different countries, according to the diversity of the oral microbiota of the snakes, which can be considered nonspecific and associated with the environment, with animal feeding habits and seasonality (11, 20).

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