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ORIGINAL ARTICLE

The identification of bacterial flora in oral cavity of snakes

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Abstract Snakebites are a great public health concern in tropical and subtropical countries. It cannot only cause poisoning but also yield some infections in victims. There are some pathogenic agents in snake's oral flora. This study was carried out to determine bacterial agents existing in the oral cavity of venomous and non-venomous snakes in Kashan, Iran. Using sterile swabs, the samples were obtained in two stages from the oral cavity of 11 venomous and non-venomous snakes before feeding and 3 weeks later. Then, they were cultured on Mac Conkey blood agar mediums. Gram staining of all samples was performed. Appropriate mediums and tests were fulfilled to determine gram-positive and gram-negative bacteria. The highest rate of infection belonged to coagulase-negative *Staphylococcus* (34.5 %) and the lowest rate was for *Pseudomonas* (3.1 %). *Salmonella* (18.8 %); *Escherichia* and *Providencia* (each 12.5 %); and *Proteus*, *Enterococcus*, and *Bacillus* (each 6.2 %) were other contributing pathogens found in snakes oral cavity. The obtained findings demonstrated significant bacterial pathogens in oral cavity of venomous and non-venomous snakes. Therefore, not only anti-venom treatment but also, due the probability of infections, the diagnosis and treatment should be considered in victims.

Keywords Bacterial flora · Oral cavity · Venomous snakes · Non-venomous snakes

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Introduction

Snakes are the members of reptile species. Snakes have close relation with alligators, tortoises, and crocodiles. Using bifurcated tongue, they can smell and trace their prey. Moreover, with the help of Jacobson's organ and nostril chamber, they augment the smell sensation. Some snakes have thermal receptors of which can aid to detect warm-blooded animals (Ismail and Memish 2003). Venomous snakes have a pair of big and hollow teeth called canine which situated at the anterior part of the upper jaw. Canine teeth have needle-like grooves containing venom originating from poison glands. Snakes inject venom into the deep body of their prey (Dehghani et al. 2012). But non-venomous snakes have simple teeth without a route to poison glands. Semi-venomous snakes are similar to non-venomous ones having the prominent distinctive characteristic of fangs or posterior grooved canine teeth. Venomous snakes usually move slowly, while semi-venomous snakes as much as non-venomous counterparts move very fast (Rose 2001). Snakes are found in most part of Iran, especially in the desert and dried regions. Sixty-nine species, out of which 36 are non-venomous, 25 venomous, and 8 semi-venomous, have been identified in Iran (Dehghani et al. 2012). More than 3500 species have been found worldwide which less than 10 % are venomous (Bawaskar 2004; Meenatchisundaram and Michael 2009; Dehghani et al. 2012). Snakebite prevalence rate, irrespective of involved species, is different from country to country and

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even region to region within one country. It depends on various parameters including climate, biological elements, environmental diversity, distribution of venomous snakes in one region, population density, economic activity, and settlement variation (Otero-Patiño 2009). It is a major public health challenge in rural areas. Although 10–20 % of the total reported snakebite cases occur at home or surrounding areas, most occurred events represent the hazard for agriculture-related professions in rural areas. Approximately, 85–90 % snakebites happen in rural and agricultural section because mice are the main food for snakes and also the vermin for agricultural products (Jorge et al. 2004; Otero-Patiño 2009; Chippaux 2010; Thomas and Brook 2011; Dehghani et al. 2012; Machado et al. 2012). Seventy percent of the snakebite sites are at extremity organs (Otero-Patiño 2009). Annually, 1.22 to 5.5 million cases, out of which 125,000 cases lead to death or disability, has been reported. Most mortality happens in the southeast part of Asia (Kasturiratne et al. 2008; Adukauskienė et al. 2010; Rita et al. 2011). Only in Asia, it is estimated that 4 million cases happen annually. Fifty percent are venomous snake attacks killing 100,000 persons every year (Sharma et al. 2004). In India, 200,000 snakebite cases have been reported annually. Out of them, 35,000–50,000 cases are the victims of venomous snakes (Chippaux 1998). From 2001–2009, there were 5000–7000 documented cases in Iran where seven to eight cases killed once a year (Dehghani et al. 2012). On the other hand farmers, cattle breeders and also veterans believe that snakebites are the common cause of mortality among pets, yielding a predominant economical loss (Silva et al. 2011). Snakebites are associated with pain and infection which may be localized or systematic. Consequently, it may cause shock, acute renal failure, coagulopathy, and heart muscle damage (Dehghani et al. 2012). Most complications caused by snakebite are due to poisoning effects such as hemorrhagic or neurotoxicogen effects which may be associated with secondary bacterial infections which are also being presented by non-venomous snakebites (Tagwireyi et al. 2001; Hejnar et al. 2007; Fonseca et al. 2009; Huang et al. 2012; Neil et al. 2012). The commonest manifestation of a snakebite infection is abscess. Some bacterial infections are expected as the main cause of mortality (Jorge et al. 2004; Fonseca et al. 2009). The species of bacteria in snakes' oral cavity are the key elements for the kind of infection (Huang et al. 2012). Like other creatures, snakes' oral cavity is a suitable place for bacterial growth some of which are as the normal oral flora. Using obtained samples from venom and the oral cavity and culturing them, some pathogenic bacteria have been identified, but some infections have secondary etiology due to snakebite. Some snake bites, especially caused by *Viperidae* and *Cobras*, can cause necrosis at the site of bite which increases the likelihood of a secondary infection (Theakston et al. 1990). The oral flora of various snake species and also different geographical ones varies (Shek et al. 2009). Even some authors believe that seasonal changes have an influence on the flora (Blaylock 2001). Various bacteria have been recognized from the snakes' cavity. The most significant ones are *Pseudomonas* and *Aeromonas* (Cooper and Leakey 1976), *Morganella morganii* (Jorge et al. 2004), *Staphylococcus aureus*, *Escherichia coli*, *Proteus*, *Colestridia*, *Enterococcus*, coagulase-negative *Staphylococcus* (Thomas and Brook 2011), *Stenotrophomonas maltophilia* (Hejnar et al. 2007), *Acinetobacter*, *Klebsiella*, and *Shigella* (Theakston et al. 1990; Fonseca et al. 2009). Bacterial infections caused by snakebite have been taken into account worldwide, but neither a comprehensive study has been carried out in this literature in Iran nor we did access to anyone at the time of the research. Given that snakebite is a great nationwide concern for public health in Iran and that it not only leads to poisoning but also causes bacterial infections, the present study was fulfilled to investigate the bacterial condition in the oral flora of venomous and non-venomous snakes.

Materials and methods

To fulfill the study, 11 venomous and non-venomous snakes collected from different regions in Kashan, Iran during 6 months were kept and fed in separate closed 12-l containers. They were fed with BALB/c mice. The sampling from the oral cavity was performed in two stages: before feeding and 3 weeks after feeding. Using sterile swabs, the samples were taken from snakes' oral cavity. Then, they were cultured on Mac Conkey and 5 % sheep blood agars. To recognize aerobic bacteria, petri dishes which contained agars were incubated for 24 h at 37 °C. The type of bacteria was determined by a microbiologist. Then, gram staining was performed for all colonies. Additionally, catalase and oxidase tests were fulfilled on bacteria. Some medium and tests were used such as Triple sugar iron (TSI), Simmons citrate agar, sulfide indole motility (SIM), urease, oxidation- fermentation test (OF), methyl red reaction, and Voges-Proskauer test (MRVP) to define gram-negative bacteria. To determine gram-positive bacteria, the medium and tests such as Mannitol salt agar, coagulase test, bile esculin agar (BE), growth in 6.5 % NaCl were used. After classification and drawing tables, the obtained data were extracted.

Results

By obtaining 20 samples from 11 venomous and non-venomous snakes and culturing them, varied bacteria were identified. Overall, eight bacteria were identified including

Table 1 The identified bacteria from cultured samples of venomous and non-venomous snakes before and after feeding

Sampling after feeding	Sampling before feeding	Snake species	Sample no.
1	*Coluber ravergieri*	*Pseudomonas* coagulase-negative *Staphylococcus*	–
2	*Spalerosophis diadema*	*Salmonella*	*Salmonella*, coagulase-negative *Staphylococcus*
3	*Vipera lebetina*	*Salmonella*	*Salmonella*, *Escherichia*
4	*Spalerosophis microlepis*	*Providencia*	*Providencia*, *Escherichia*
5	*Coluber rhodorachis*	Coagulase-negative *Staphylococcus*	Coagulase-negative *Staphylococcus*
6	*Coluber ravergieri*	*Salmonella* coagulase-negative *Staphylococcus*	–
7	*Spalerosophis diadema*	*Proteus* coagulase-negative Staphylococcus	*Proteus*, coagulase-negative *Staphylococcus*
8	*Coluber rhodorachis*	*Enterococcus*	*Enterococcus*, *Salmonella*
9	*Coluber karelini*	*Bacillus*	*Bacillus*, coagulase-negative *Staphylococcus*
10	*Coluber ravergieri*	*Escherichia* coagulase-negative *Staphylococcus*	*Escherichia*, coagulase-negative *Staphylococcus*
11	*Vipera lebetina*	*Providencia*	*Providencia*, coagulase-negative *Staphylococcus*

Pseudomonas, *Salmonella*, *Proteus*, *Staphylococcus*, *Enterococcus*, *Bacillus*, *Escherichia*, and *Providencia*. At the second stage, due to the death of two snakes, the sampling was not performed from them (Table 1). The results demonstrated that the most and the least common contributing pathogens were coagulase-negative *Staphylococcus* (34.5 %) and *Pseudomonas* (3.1 %), respectively. *Salmonella* (18.8 %), *Escherichia*, and *Providencia* (each 12.5 %), and *Proteus*, *Enterococcus*, and *Bacillus* (each 6.2 %) were other identified pathogens (Table 2). There was no difference regarding the oral flora before and after feeding, i.e., the flora was the same in more than 90 % of the cases. Out of 11 captive snakes, 9 were non-venomous and the rest (2) were venomous being *Vipera lebetina* species. All eight abovementioned pathogens were found in nine non-venomous snakes. Non-venomous species were *Salperosophis diadema* (2 cases), *Coluber ravergieri* (3), *Coluber rhodorachis* (2), *Spalerosophis microlepis* (1), and *Coluber karelini* (1). Two *V. lebetina* species caused infection due to *Salmonella*, *Staphylococcus*, *Escherichia*, and *Providencia*.

Discussion and conclusion

In the current study, some contributing pathogens causing infection were identified in snakes such as *Pseudomonas*, *Salmonella*, *Proteus*, *Staphylococcus*, *Enterococcus*, *Bacillus*, *Escherichia*, and *Providencia*. The most common and the least pathogenic were coagulase-negative *Staphylococcus* and *Pseudomonas*, correspondingly. Other found pathogens included *Salmonella*, *Escherichia*, and *Providencia*, *Proteus*, *Enterococcus*, and *Bacillus*. Snakes have no feet or hands for defence; therefore, they attack the enemies with mouth using their teeth. In addition to poisoning due to snakebites caused by venomous snakes and injecting venom via their fangs,

Table 2 The recognized bacteria from cultured samples of the oral cavity of venomous and non-venomous snakes

Bacterial species	Before feeding	After feeding	Venomous snakes	Non venomous snakes	Case number	Percent bacterial species
Coagulase-negative *Staphylococcus*	5	6	+	+	11	34.5
Salmonella	3	3	+	+	6	18.8
Providencia	2	2	+	+	4	12.5
Escherichia	1	3	+	+	4	12.5
Bacillus	1	1	–	+	2	6.2
Proteus	1	1	–	+	2	6.2
Enterococcus	1	1	–	+	2	6.2
Pseudomonas	1	–	–	+	1	3.1
All of them	8	7			32	100

(+) Positive: recognized bacteria from oral cavity

(–) Negative: not recognized bacteria from oral cavity

there is the likelihood of infection caused by pathogens found in their fangs or teeth. As the study results revealed that there was a wide spectrum of pathogens in the oral cavity of the studied snakes whose bites may cause not only poisoning but also infection which exacerbates the condition in victims. In a study on bacteria found in the oral flora of North American rattle snakes, they found coagulase-negative *Staphylococcus*, *Proteus* sp., and *Pseudomonas* sp. (Goldstein et al. 1979). Another study performed on non-venomous snakes showed coagulase-negative *Staphylococcus*, *Acinetobacter* sp., *Hafnia alvei*, *Arizona hinshawii*, *Salmonella* sp., *Shigella* sp., *Klebsiella oxytoca*, and *Pseudomonas aeruginosa* (Fonseca et al. 2009). Researchers found more than 50 bacteria species in the oral flora of Chinese Cobra including *Aeromonas*, *Proteus*, *Colestridium* sp., and also *Staphylococcus aureus*, *Enterococcus*, and coagulase-negative *Staphylococcus* (Shek et al. 2009). In a study carried out in Hong Kong, the researchers found 72 bacteria in venomous and non-venomous snakes including gram-positive and gram-negative and also anaerobic bacteria (Lam et al. 2010). The bacteria species are the determinate factor for snakebite-caused infection (Huang et al. 2012). In a study conducted in Brazil, the researchers isolated some bacteria, mostly *M. morganii* and other enteric bacillus from abscess drainage, the oral cavity, canine teeth, and snakes' venom (Jorge et al. 2004). The secondary infection is one of snakebite complications. The isolated pathogens from abscess site encompassed *Pseudomonas* sp., *Proteus* sp., *Escherichia coli*, *Providencia*, and *M. morganii* (Theakston et al. 1990). Some studies showed that there were severe infections caused by *Aeromonas hydrophila* or *Vibrio vullnificus* after snakebite (Cooper and Leakey 1976; Jorge et al. 1998, Huang et al. 2012). As it is obvious in the studies conducted by other researchers, various bacterial pathogens can be found in the oral cavity. This finding is compatible with our findings. It is worthwhile to mention that not only the treatment of poisoning caused by venomous snakebites but also the probability of infections caused by venomous and non-venomous snakebites should be considered in victims. *P. aeruginosa* being a pathogen causing infection among the Kenyan population and also a prevalent pathogen among captive reptiles is a great concern for man's health. *Pseudomonas* species belonging to *Enterobacteriaceae* can cause local infections in snakes as necrosis in the oral cavity and also ultimately septicemia (Cooper and Leakey 1976). Hence, in the case of exposure to animal or captive reptile bite, the probability of infections should be considered. The study confirmed the involved bacterial infection caused by some pathogens found in snake's oral flora. The current finding has been mentioned in other studies performed in other countries. In most studies, except few ones, researchers have focused on the infections caused by venomous snakes. Bearing in mind non-venomous snakebite and the probability of infection in human, it is necessary to monitor the victim's condition very carefully. With taking into account the high prevalence rate of snake bite partly caused by non-venomous snakes in Iran annually; in the case of exposure to snake venom, moreover to anti-venom treatment, the care providers should assess and treat the wound infections.

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Conflict of interest The authors declared that they have no competing interests.

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