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Normal oral bacterial flora from some southern African snakes

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ABSTRACT

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Eighteen snakes representing 11 species were subject to mouth swabbing on 58 occasions. Of these swabs, 52.2 % were positive for bacteria. A total of 92 bacterial isolates were cultured, representing 30 species of which 81.5 % were Enterobacteriaceae, 16.3 % Gram positive cocci, and 2.2 % anaerobes. Swabs from non-venomous snakes were more commonly bacteriologically sterile than those from venomous snakes ($P = 0.0107$). The oral bacterial flora did not differ between captive and newly captured snakes. The bacterial species found were not constant in a single snake with time, in the same snake species, the same serpentarium or geographically. The bacteria most commonly cultured were *Proteus* spp., *Pseudomonas* spp., *Salmonella arizonae* and *Staphylococcus epidermidis*. Colony counts tended to be low. Three or more bacterial species per venomous snake per occasion were more common in winter than summer ($P = 0.0192$).

Keywords: Oral bacterial flora, *Proteus* spp., *Pseudomonas* spp., *Salmonella arizonae*, snakes, *Staphylococcus epidermidis*

INTRODUCTION

Sepsis in human snakebite victims in southern Africa occurs if there is an associated haematoma or necrosis at the bite site (Blaylock 1999). This concurs with the findings on Malayan viper bites (Reid, Thean, Chan & Baharom 1963). Bacteria were cultured from 14 closed necrotic areas, abscesses or haematomas from human snakebite victims in KwaZulu-Natal. At the time of surgery the exudate was odourless in all cases (Blaylock 1999). *Proteus* spp., *Morganella morganii, Serratia* spp., *Serratia liquifaciens, Escherichia coli, Citrobacter diversus, Citrobacter freundii, Enterobacter agglomerans, Salmonella arizonae, Streptococcus pyogenes* and *Staphylococcus* spp. were the only bacteria isolated. The origin of these bacteria could be the victim's skin, the snake's oral cavity or the injected venom. Studies on the bacterial flora in snakes' mouths and venom have been undertaken in other countries but not South Africa (Goldstein, Citron, Gonzalez, Russell & Finegold 1979; Arroyo, Bolaños & Muñoz 1980; Soveri & Seuna 1986; Jorge, De Mendonça, Ribeiro, Da Silva, Kusano & Cordeiro 1990; Theakston, Phillips, Looareesuwan, Echeverria, Makin & Warrell 1990). This study was undertaken to determine the numbers and species of bacteria occurring in the oral cavities of South African snakes.

MATERIALS AND METHODS

During 1991 and 1992, 11 snakes representing seven species, were captured in KwaZulu-Natal. Their oral cavities were swabbed for bacteriological culture and the swabs submitted to Eshowe Hospital. The mouths of all of them were swabbed once, with two being swabbed on a later occasion, giving a total of 13 swabs.

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In Gauteng, the study was continued during 1994 and 1995 in a similar way at Gold Fields West Hospital (GFWH) in Westonaria, and at Leslie Williams Memorial Hospital in Carletonville. Each snake was swabbed twice, one swab being submitted to the bacteriological department of each hospital. Eleven snakes comprising nine species were used in this study, four of which were newly captured. These snakes included the puff adder (*Bitis arietans*) and the three house snakes (*Lamprophis* spp.) from KwaZulu-Natal. All were swabbed on repeat occasions over a six to eight month period resulting in ninety swabs over 45 occasions.

All three hospitals were of second level status with consequent microbiological limitations, and outside help was occasionally sought for bacterial identification.

Snakes and serpentaria

All the snakes that were swabbed were healthy. In Gauteng, they were housed under three different conditions in the same building. Three puff adders, a Gaboon viper (*Bitis gabonica*) and a python (*Python natalensis*) were kept in a room. A rinkhals (*Hemachatus haemachatus*), a Mozambique spitting cobra (*Naja mossambica*) and a rhombic night adder (*Causus rhombeatus*) were housed in a glass container previosuly used as an aquarium, and three species of house snakes, (*Lamprophis fuliginosis*, *Lamprophis guttatus*, and *Lamprophis inornatus*) were accomodated in a wooden box.

Swabs

These were of the standard type containing a transport medium. For the samples collected in Eshowe Transystem Amies swabs (Copan, Italy) were used and in Gauteng the Amies Transport swabs (Clinical Sciences, Johannesburg).

Swabbing procedure

On each occasion, an individual snake was captured by one person who opened the mouth using a sterile instrument while a second person rotated the swab on the floor of the mouth between the larynx and mandibular teeth. If a swab was deemed to be contaminated in any way, it was immediately discarded. Bacteriological examination commenced within a few minutes as the swabbing was undertaken within the grounds of, or within each hospital.

Aerobic and anaerobic culture

The swabs were directly inoculated onto culture plates which were incubated both aerobically and anaerobically at 37 °C .

Aerobic cultures on MacConkey's agar without crystal violet, 5 % horse blood agar and cooked meat broth (South African Institute for Medical Research, Johannesburg) were maintained for 24 hours. At GFWH in the absence of growth after 24 h, all plates were reincubated for a further 24 h, and the cooked meat broth was subcultured onto 5 % blood agar and McConkey's agar. If *Haemophilus* spp. was suspected from Gram stain, bacitracin-agar was utilized.

Anaerobic cultures (Gas Generating Kit Anaerobic System, Oxoid Ltd, England) on 10 % blood agar (not prereduced) were maintained for 48 h. Any growth was subject to an aerotolerance test by subculturing onto two 5 % blood agar plates for aerobic and carbon dioxide jar incubation (Gas Generating Kit Carbon Dioxide System, Oxoid Ltd, England), and onto 10 % blood agar for further anaerobic incubation.

Gram staining and colony counts

Immediately after inoculation, smears were prepared from the same swabs on glass slides for microscopic examination. These were stained with Gram's stain.

Colony counts of any bacterial growth on solid media were graded after 24 h as follows:

- trace, less than 10 colonies
- +, 10–50 colonies
- ++, 50–100 colonies
- +++, more than 100 colonies per plate.

Bacterial identification

The Streptex test (Murex. Biotech Ltd, England) was used for Streptococci. The *Staphylococcus* tube coagulase test was used for Staphylococci at Eshowe, (Staphaurex, Murex Biotech Ltd, England) and the Oxoid staphylase test (Oxoid Ltd, England) was used in Gauteng.

Gram negative aerobic rods were identified in Eshowe with the aid of Microbact 24A + E (Disposable Products Pty, Ltd, Adelaide, Australia) and in Gauteng with the Analytical Profile Index 10 S (Biomérieux sa, France).

Anaerobes were identified with the Rapid ID 32A (Biomérieux sa France).

Statistical analyses

A computerized verson of the Fisher's exact test (Instat 3 Statistics Package, GraphPad Software, San Diego, California, USA) was used to analyze the results.

RESULTS

KwaZulu-Natal (Table 1)

Fifteen bacterial species were isolated, of which 80 % were Enterobacteriaceae, 20 % Gram-positive aerobic cocci with a further slightly fusiform Gram-negative organism which was visualized but not cultured.

TABLE 1 Bacterial species isolated from the snakes in KwaZulu-Natal

Snake	Date	Bacteria
Psammophis brevirostris (stub-nosed grass snake)	Dec. 1991 Jan. 1992	*Acinetobacter lwoffii* *Pseudomonas stutzeri* *Pseudomonas* spp.
Telescopus semiannulatus (tiger snake) Snake 1	Dec. 1991	*Aeromonas caviae* *Aeromonas hydrophila* *Staphylococcus aureus*
Snake 2	Dec. 1991	*Escherichia coli* *Acinetobacter lwoffii* *Serratia* spp.
Naja mossambica (Mozambique spitting cobra) Snake 1		*Klebsiella pneumoniae* *Staphylococcus epidermidis*
Snake 2		*Staphylococcus* spp.
Snake 3		*Klebsiella oxytoca* *Enterobacter agglomerans*
Snake 4		*Enterobacter agglomerans* *Staphylococcus* spp.
Bitis arietans (puff adder)	Dec. 1991 Apr. 1992	*Citrobacter freundii* *Salmonella arizonae* *Staphylococus aureus* No growth
Lamprophis guttatus (spotted house snake)		No growth
Lamprophis fuliginosis (brown house snake)		Scanty slightly fusiform Gram-negative bacteria No growth
Lamprophis inornatus (black house snake)		No growth

All initial swabs were on newly captured snakes

Gauteng (Tables 2–4)

Twenty-two bacterial species were isolated, of which 81.8% were Enterobacteriaceae, 13.6% Gram-positive aerobic cocci and one anaerobe (4.5%). There was one further visualized but uncultured Gram-negative bacillus.

Bacteria identified at the two hospitals in Gauteng were identical in 53.3% of the cases, similar in 22.2% and totally dissimilar in 6.7%.

DISCUSSION

Bacterial culture was undertaken at 37 °C as this is normal human body temperature. Arroyo *et al.* (1980) and Theakston *et al.* (1990) cultured at 37 °C , while Goldstein *et al.* (1979) utilized a temperature of 35 °C. In similar studies by Soveri & Seuna (1986) plates were cultured in parallel at 20 °C and 37 °C . It appears that some bacteria will grow at 20 °C while all of them will grow at 37 °C . It is possible that the capability of growth at the lower temperature may be partly responsible for the bacterial species causing stomatitis in snakes (Soveri & Seuna 1986). In the studies conducted in KwaZulu-Natal and Gauteng 86.7% of the 30 identified species were Enterobacteriaceae, 10% Gram-positive aerobic cocci with one anaerobe *(Clostridium sordellii).* Of the isolates, 79.8% were Enterobacteriaceae, 16% Gram-positive cocci, 2.1% anaerobes with 2.1% unidentified.

There was some discrepant information from the two hospitals in the Gauteng study, probably explained by low bacterial numbers in the snakes' mouths. In addition, a swab rotated between the larynx and mandibular teeth is sampling a small percentage of the buccal cavity surface area. Further, Soveri & Seuna (1986) found no correlation betwen aerobic bacteria cultured from the mouth and proximal oesophagus

TABLE 2 Oral bacterial flora in non poisonous snakes in Gauteng

Gold Fields West Hospital			Leslie Williams Memorial Hospital
Date	Microscopy	Bacteria cultured	Bacteria cultured
Lamprophis fulinginosis (brown house snake) [a]			
1994.07.11	Scanty gram-negative bacilli	Nil	Nil
1995.01.23	Nil	Nil	*Staphylococcus aureus*
Lamprophis guttatus (spotted house snake)[a]			
1994.07.11	Nil	Nil	Nil
1995.01–23	Nil	Nil	Nil
Lamprophis inornatus (black house snake)[a]			
1994.07.11	Nil	Nil	Nil
1995.01.23	Scanty gram-negative bacilli	*Proteus* spp.	Nil
1995.02.14	Nil	Nil	Nil
Python natalensis (python) Swazliand[b]			
1995.01.23	Nil	Nil	Nil
1995.03.30	Not done	*Pseudomonas* spp.	*Stenotrophomonas maltophilia Xanthomonas* spp. *Enterobacter aerogenes*
1995.05.04	Nil	Nil	Nil
1995.06.12	Nil	Nil	Nil
1995.08.10	Nil	Nil	Nil

[a] Wooden box
[b] Room

of the same snake on the same occasion, although when the flora of both locations were compared in the whole snake population, it was similar.

Geographical variation is possible, as only five of 12 and 17 bacterial species isolated in KwaZulu-Natal and Gauteng, respectively, were identical. In KwaZulu-Natal three bacterial species at two swabbing intervals were cultured from the puff adder. When transferred to Gauteng, after a three year interval, and after five swabbings, one organism was re-cultured (*Salmonella arizonae*) while four species of Enterobacteriaceae were cultured for the first time from the same snake. This phenomenon has been shown previously (Theakston *et al.* 1990), but is disputed by Arroyo *et al.* (1980).

There is little difference in quality and quantity of bacteria isolated from newly captured and captive snakes. The initial swabs of five out of 15 newly captured snakes were negative for bacteria, while seven out of 13 captive snakes, initially positive for bacteria, became temporarily negative. Theakston *et al.* (1990) found a wider range of bacteria (but no *Corynebacterium* spp.) and more positive cultures in newly captured snakes, while Arroyo *et al.* (1980), found a heavier bacterial load with no difference in bacterial species in captive snakes. These discrepancies may be due to environmental bacterial load and the mouth cleansing abilities of the snakes. This latter is suggested by this study, where there is variation in quality and quantity of bacteria in snakes sharing the same serpentarium.

Non-venomous snakes' mouths were sterile on ten out of 15 occasions (66.7 %) as opposed to 11 out of 43 occasions (25.6 %) in venomous snakes ($P = 0.0107$). These negative results may be due to insensitive culture techniques although in the present study, where microscopy was performed, little additional information was forthcoming. It may be that a low environmental bacterial load of the wooden box

TABLE 3 Oral bacterial flora in *Bitis arietans in Gauteng*

Date	Gold Fields West Hospital			Leslie Williams Memorial Hospital		
	Microscopy	Colony count	Bacteria cultured count	Microscopy	Colony count	Bacteria cultured
Snake 1— Gauteng						
1995.02.21[a]	Nil		Nil	Nil		Nil
1995.03.30	Not done		*Stapylococcus aureus*	Not done		*Staphylococcus*
1995.05.04	Not done		Nil	Not done		Nil
1995.06.12	Nil		Nil	Nil		Nil
1995.08.10	Not done	+	*Salmonella arizonae*	Not done		Nil
Snake 2—Zimbabwe						
1995.01.23[a]	Nil		Nil	Not done		Nil
1995.02.14	Nil		Nil	Not done		Nil
1995.03.30	Not done		*Pseudomonas* spp.	Not done		Nil
1995.05.04	Nil		Nil	Not done		Nil
1995.06.12	Nil		Nil	Nil		Nil
1995.08.10	Mixed organisms	++	*Pseudomonas aeruginosa*	Not done		Nil
Snake 3—KwaZulu Natal						
1995.01.23	Scanty gram negative bacilli	+	*Pseudomonas* spp.	Not done		*Proteus mirabilis* *Pseudomonas aeruginosa*
1995.02.14	Nil	Trace	*Pseudomonas* spp. *Proteus* spp.	Not done		*Pseudomonas aeruginosa*
1995.03.30	Not done		*Escherichia coli*	Not done		*Escherichia coli.* *Proteus vulgaris*
1995.05.04	Not done	+	*Pseudononas aeruginosa*	Not done	Trace	*Salmonella arizonae*
1995.06.12	Not done	++	*Pseudomonas aeruginosa*	Not done	+++	*Pseudomonas aeruginosa* *Proteus mirabilis*

[a] Newly captured

All were housed in the same room

containing the three non-poisonous house snakes affected this result. However, if the python is compared with the four poisonous snakes sharing the same room, then the negative cultures were 60 % and 36 % respectively. It is possible that the saliva of non-poisonous snakes which thinly coats the buccal cavity, is antibacterial. Jansen (1983), found that Duvernoy's gland secretions were antibacterial and it is known that venom of South African snakes has antibacterial properties (Blaylock 2000a). Venom probably does not always coat the buccal surface as saliva does, which may explain this discrepancy. This antibacterial property of venom probably originated as a conditioner of dental surfaces (Gans 1978).

There is no consistency of bacterial flora in the same snake since the same bacterium (*Proteus vulgaris*) was grown on all occasions only from the rinkhals. The same bacterium was grown from two snakes on four out of five, and three out of five occasions, respectively, while three of seven snakes swabbed on five or more occasions failed to grow the same organism more than once. The Gaboon viper, which produced the most bacterial species (11 over six

TABLE 4 Oral bacterial flora in *Bitis gabonica, Causus rhombeatus, Hemachatus haemachatus* and *Naja mossambica* in Gauteng

Date	Gold Fields West Hospital			Leslie Williams Memorial Hospital		
	Microscopy	Colony count	Bacteria cultured	Microscopy	Colony count	Bacteria cultured
Bitis gabonica (gaboon viper)[b]						
1995.01.23	Scanty gram negative bacilli		*Proteus* spp.	Not done		*Staphylococcus epidermidis* *Proteus mirabilis*
1995.02.14	Nil		Nil	Not done		Nil
1995.03.30	Not done		*Pseudomonas* spp.	Not done		*Pseudomonas aeruginosa* *Clostridium sordellii*
1995.05.04	Not done	+ + +	*Morganella morganii*+ *Stenotrophomonas maltophilia* *Pseudomonas vesicularis*	Not done	+ +	*Providentia stuartii* *Morganella morganii*
1995.06.12	Moderate gram negative bacilli	++ ++	*Providencia rettgeri* *Salmonella arizonae*	Scanty gram negative bacilli	+++	*Salmonella arizonae* *Proteus mirabilis* *Clostridium sordellii*
1995.08.10	Some mixed organisms		*Tatumella ptyseos*	Not done	+ +++	*Salmonella arizonae* *Providentia rettgeri*
Causus rhombeatus (rhombic night adder)[c]						
1995.02.21[a]	Nil		Nil	Nil		Nil
1995.03.30	Nil		Nil	Not done		Nil
1995.05.04	Not done	+ +	*Staphylococcus epidermidis* *Pseudomonas vesicularis*	Not done	Trace Trace	*Staphylococcus epidermidis* *Salmonella arizonae*
1995.06.12	Not done	+	*Salmonella arizonae*	Nil	Trace	*Salmonella arizonae*
1995.08.10	Nil		*Salmonella arizonae*	Not done	++	*Salmonella arizonae*
Hemachatus haemachatus (rinkhals)— Gauteng[c]						
1995.02.14[a]	Scanty gram negative bacilli	Light growth	*Proteus* spp.	Not done		*Proteus vulgaris*
1995.03.30	Mixed organisms		*Proteus* spp. *Staphylococcus epidermidis*	Not done		*Proteus vulgaris*
1995.05.04	Not done	+ +	*Proteus vulgaris* *Staphylococcus epidermidis*	Not done	Trace + +	*Proteus vulgaris* *Staphylococcus epidermidis* *Stenotrophomonas maltophilia*
1995.06.12	Not done	+	*Proteus vulgaris*	Nil		*Proteus vulgaris*
1995.08.10	Mixed organisms	+++ +++	*Citrobacter freundii* *Providencia rettgeri*	Not done	+ +++ Trace	*Escherichia coli* *Proteus vulgaris* *Acinetobacter calcoaceticus*
Naja mossambica (Mozambique spitting cobra)—Mpumalanga[c]						
1995.06.22	Not done	+	*Salmonella artizonae*	Not done	+ ++ +++	*Salmonella arizonae* *Moraxella* spp. *Staphylococcus epidermidis*

[a] Newly captured
[b] Room
[c] Glass container

occasions), did not harbour the same bacterium more than twice.

Snakes sharing the same serpentarium for several months did not necessarily harbour the same bacteria. The room serpentarium housed two snakes which frequently produced growth of multiple bacteria, whilst three frequently yielded low bacterial numbers or no growth at all. This suggests that buccal bacterial species and numbers are not totally dependent on the environmental bacterial load. In addition, snakes of the same species do no necessarily harbour the same bacterial flora and numbers, as shown by three puff adders which shared the same housing.

Oral bacterial flora of snakes may vary seasonally. It is known that snake venom composition can vary in the same snake over a year. (Williams & White, 1992). Results from the long term captive venomous snakes show that 0.9 bacterial species per occasion were cultured in summer and 1.8 species per occasion in winter, while the largest colony counts (venomous snakes) were also recorded at this time of the year. It was only in winter that three or more bacterial species at a time were cultured form venomous snakes. This occurred on six out of 17 occasions in winter as opposed to nill out of 15 occasions in summer ($P = 0.0192$). The activity of fresh cobra venom against anaerobic bacteria diminishes during winter (Blaylock 2000a) and the severity of clinical cytotoxicity in bitten human patients is reduced during this period (Blaylock 2000b). The diminution of venom potency in winter could explain these differences.

The bacteria cultured during this study are similar to those described from *Bothrops jararaca* from Brazil (Jorge *et al.* 1990) where fang sheath and venom were cultured, and from *Calloselasma rhodostoma* from Thailand (Theakson *et al.* 1990).

Goldstein *et al.* (1979), however, found 32.6% of the oral bacterial flora to be anaerobic comprising several *Clostridium* spp., *Bacteroides fragilis* and *Propionibacterium acnes* in rattle snakes. Arroyo *et al.* (1980) cultured the venom and mouth cavities of Costa Rican snakes. Ninety-six isolates were Gram-negative Enterobacteriaceae while 63 were Clostridia comprising eight species. That these two studies yielded such a high number of anaerobic bacteria could be explained by the culture of venom and not solely buccal mucosa, geographic variation of snake oral flora and the use of better anaerobic culture techniques. However, 14 closed necrotic areas, abscesses or haematomas in snake bitten patients, at the time of surgery, yielded pus without the foul smell of anaerobic sepsis and 18 Enterobacteriaceae, a single *Staphylococcus* sp. and *Streptococcus pyogenes* were cultured (Blaylock 1999). There are no published cases of tetanus or clostridial myonecrosis following snakebite in southern Africa.

In studies where the buccal cavity and venom have been cultured separately the buccal cavity produced a higher bacterial load (Goldstein *et al.* 1979; Arroyo *et al.* 1980; Theakston *et al.* 1990).

Snakes do not appear to have permanent oral bacterial flora. Rather they are transient and probably of environmental origin as suggested by Soveri & Seuna (1986). The same authors found neither a correlation between the oral and oesophageal aerobic bacterial flora of individual snakes sampled on the same occasion nor between bacterial flora and prey type, time of feeding or time of swabbing. When a snake is awake, and especially when it is on the move, it frequently flicks its tongue in and out to explore the environment. This, together with feeding and drinking must inoculate the buccal cavity with bacteria. Occasionally swallowed prey will defecate before or during swallowing, temporarily giving a heavy bacterial load which is probably mostly cleared by a self cleansing mechanism in the buccal cavity in which Duvernoy's gland secretions and venom play a role.

There is good correlation between bacteria isolated from infected snakebite sites in humans in southern Africa (Blaylock 1999) and this study, where Gram-negative Enterobacteriaceae predominate and anaerobic bacteria are uncommon.

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