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THE BACTERIAL FLORA OF VENOMS AND MOUTH CAVITIES OF COSTA RICAN SNAKES[1]

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Bacterial flora isolated from the venoms and mouth cavities of Costa Rican snakes suggest that secondary bacterial infection following snakebite is a real possibility. No one treatment strategy can be expected to completely control all infections; but the results suggest that in emergency situations administration of penicillin combined with a broad-spectrum antibiotic such as chloramphenicol or tetracycline could prove effective.

Introduction

Snakebites frequently induce severe bacterial infections that require antibiotic therapy (Rosenfeld, 1971). The responsible microorganisms may come from the natural flora of the victim's skin, from the environment, or from the venom, fangs, or mouth cavity of the offending snake (Ledbetter and Kutscher, 1969). However, most research on snake venom and mouth cavity microorganisms has been carried out in localities with a snake fauna very different from that found in Costa Rica, and has often employed outmoded laboratory procedures incapable of isolating fastidious anaerobes (Christiansen, 1955; Ledbetter and Kutscher, 1969; Parrish et al., 1956; Williams et al., 1934).

Only one Costa Rican paper dealing with microorganisms in venoms has been published (Brunker and Fernández, 1974). This work, which confined itself to anaerobic bacteria, was performed with pools of lyophilized venoms prepared without taking sterile precautions. Thus, some of the microorganisms isolated could have been introduced from the environment.

The present study employed modern culture techniques for isolating anaerobic organisms and used venoms obtained in a manner calculated to minimize bacterial contamination. The resulting data reported here relate to the isolation and the sensitivity to antibiotics of the aerobic and anaerobic bacteria found in individual fresh venoms and in mouth swabs from recently captured *Bothrups asper* (fer-de-lance), *Lachesis muta* (bushmaster), and *Crotalus durissus* (Central American rattlesnake). In terms of bite frequency and venom lethality, these are the most important snakes in Costa Rica (Bolaños, 1971). Information is also presented on the number of viable aerobic bacteria found in fresh venoms as compared to venoms from snakes of the same species that had undergone visible physical alterations—being white in color (with mucus) and containing a prurulent precipitate.

Materials and Methods

All the venoms—both fresh ones from recently captured snakes and altered ones from captive snakes—were expressed directly from the fangs into appropriate sterile culture media, the fangs having first been rubbed with sterile cotton swabs and a few drops of venom discarded to clean the venom ducts. Three to four drops of venom were deposited

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in 5 to 10 ml of broth; and in those cases where recovery of anaerobic bacteria was attempted, the tubes were exposed to a gentle stream of oxygen-free CO_2 at the time of inoculation in order to maintain anaerobic conditions. The bacterial flora of both venom and mouth cavities (including the fang sheaths and oropharynx) were studied by rubbing sterile cotton swabs on the appropriate anatomic regions and culturing those swabs.

The media used for culturing anaerobic bacteria were those recommended by the Virginia Polytechnic Institute's Anaerobe Laboratory (Holdeman et al., 1977). Initially, prereduced peptone-yeast extract-glucose broth (PYG) in screw-cap tubes was employed, but in later experiments prereduced chopped meat-glucose (CMG)—in tubes sealed with rubber stoppers—was used to improve the chances of isolating fastidious anaerobes. Following 24 to 48 hours of incubation at 37°C, the cultures were plated out onto blood agar medium in Brewer jars under anaerobic conditions (GAS PAK®) and were incubated for 24 and 48 hours at 37°C. The resulting colonies were then transferred to two slants (of CMG plus agar), one of which was subjected to an aerotolerance test. Spore-producing strains were also subjected to thermal shock (Moore and Holdeman, 1972). All the strains isolated were sent to the Virginia Polytechnic Institute Anaerobe Laboratory for final identification and to test their sensitivity to antibiotics.

Aerobic flora were cultured from both fresh and altered venoms in brain-heart infusion broth for 24 hours at 37°C, and were then plated on blood agar, McConkey agar, and *Salmonella* and *Shigella* agar. Gram-positive cocci were identified by their morphology, hemolytic activity, fermentation reactions, and coagulase activity (Bailey and Scott, 1966). Gram-negative rods were identified using the biochemical tests recommended by Edwards and Ewing (1972). Using commercial discs, all strains were tested for sensitivity to a range of antibiotics. Sixteen samples of fresh venoms, as well as six samples of altered venoms from captive snakes, were used for aerobic colony counts on blood agar plates (Gandy et al., 1963).

Results

No qualitative differences were observed between the bacterial flora obtained from fresh venoms and those obtained from the mouth cavities of the three snake species studied, even when the snakes came from disparate geographic areas with very different ecological features. The only anaerobes isolated—even with media and techniques designed to detect fastidious organisms—were gram-positive spore-forming rods belonging to the genus *Clostridium* (Tables 1 and 2). Only *Cl. bifermentans* was isolated when PYG broth was used. However, many more clostridial species were isolated in CMG

Table 1. Effects of culture media on the isolation of *Clostridium* species from fresh (normal) venoms and mouth cavities of Costa Rican snakes.

Experiment number	Source	Culture media	Number of samples	Positive		Number of isolates
				Samples	%	
1	Venoms	PYG[a]	42		(24)	10[c]
2	Venoms	CMG[b]	9		(89)	21
3	Mouth cavities	CMG[b]	11		(100)	32

[a] Peptone-yeast extract-glucose broth in screw-cap tubes.
[b] Chopped meat-glucose in tubes sealed with rubber stoppers.
[c] *Clostridium bifermentans* only.

broth. These species were subsequently identified and their relative frequency was determined (see Table 2). All 63 isolates were sensitive to penicillin; 94 to 98 per cent were sensitive to chloramphenicol, erythromycin, and tetracycline; and 87 per cent were sensitive to clindamycin.

Fifty-eight snakes examined for aerobic microflora yielded 102 isolates. These strains were classified as belonging to eight species, with gram-negative rods predominating over gram-positive cocci (Table 3). The gram-positive cocci were coagulase-negative and appeared, from their biochemical reactions, to be non-pathogenic micrococci. All the isolates were examined for sensitivity to ampicillin, carbenicillin, cephalosporin, chloramphenicol, erythromycin, gentamycin, kanamycin, penicillin, polymixin, streptomycin, and tetracycline. All were sensitive to chloramphenicol, gentamycin, kanamycin, and tetracycline, while showing varying degrees of resistance to the others. These results indicate that no one antibiotic will suffice for treating all the possible anaerobic and aerobic infections arising from snakebites.

In addition, colony counts obtained from 22 cultured samples of both fresh and altered *B. asper* and *L. muta* venoms are shown in Table 4. As can be seen, the altered venoms from each species yielded considerably higher colony counts than the fresh (normal) venoms. At the same time, altered *B. asper* venoms were found to yield a considerably higher count than altered *L. muta* venom, and fresh *B. asper* venoms were likewise found to yield a considerably higher count than fresh *L. muta* venoms.

Table 2. Frequency of isolation of different *Clostridium* species from fresh (normal) venoms and mouth cavities of Costa Rican snakes.

Clostridium species isolated	Snake species tested			Total No. of isolates
	Bothrops asper	*Lachesis muta*	*Crotalus durissus*	
Cl. bifermentans	9	6	4	19
Cl. butylicum	3	2	4	9
Cl. glycolicum	0	3	6	9
Cl. novyi (type A)	3	1	4	8
Cl. sporogenes	2	2	4	8
Cl. cadaveris	1	2	3	6
Cl. hastiforme	1	0	1	2
Cl. limosum	1	1	0	2

Table 3. Aerobic microorganisms isolated from 58 mouth cavity swabs or venom samples.

Type of microorganism	No. of isolates
Gram-negative rods (total)	96
Proteus vulgaris	17
Proteus morganii	15
Proteus rettgeri	8
Proteus mirabilis	4
Escherichia coli	12
Providencia sp.	16
Klebsiella group	14
Pseudomonas sp.	10
Gram-positive cocci	6
Micrococcus sp.	6

Table 4. Colony counts of aerobic bacteria isolated from Costa Rican snake venoms.

Snake species	No. of venom samples	Condition of venoms	Average No. of colonies per ml of venom	Overall range of colony counts per ml of venom
Bothrops asper	11	Normal	2×10^6	5.4×10^4 to 4.2×10^6
Bothrops asper	5	Altered	1.6×10^8	3×10^6 to 5×10^8
Lachesis muta	5	Normal	1.5×10^4	5×10^3 to 4.8×10^4
Lachesis muta	1	Altered	3×10^6	

Discussion

We have shown that the crotalid venoms tested have an abundant bacterial flora consisting mainly of clostridia and the enterobacteriacae. Most previously published work has involved preserved or pooled venoms, and must therefore be considered primarily qualitative. The quantitative data presented here were obtained by working with venoms expressed directly from individual snakes into test media.

The results show that normal (fresh) venom from *B. asper,* our most deadly and aggressive snake, may contain as many as 2×10^6 organisms per ml. This high bacterial content is important—not only clinically, but also because such large numbers of organisms may have an effect during the processing of venoms used for biochemical, pharmacologic, or immunologic research. It should therefore be borne in mind when studying snake venom that the product obtained, even under the best conditions, may not exactly correspond to the initial glandular secretion.

In examining snakebite victims, it is not easy to differentiate between the effects of bacterial infection and those of the venom itself, although there are well-documented reports of concomitant infections. An unpublished review of 85 snakebite cases admitted to Costa Rica's San Juan de Dios Hospital between 1972 and 1975 reported that 19 per cent of the patients had detectable bacterial infections. Unfortunately, very few of the infections were cultured, and none were cultured with techniques suitable for detecting anaerobic bacteria.

As this suggests, there is in snakebite an appreciable risk of bacterial infection that complicates the primary problem of envenomization. Since we feel all snakebite accidents should be regarded as potentially capable of producing both aerobic and anaerobic bacterial infections, we consider antibiotic therapy imperative. The data presented here, together with other data in the literature (Brunker and Fernández, 1974; Ledbetter and Kutscher, 1969), suggest that no one treatment strategy can be expected to completely control all infections. To do so it would be necessary to isolate the responsible organisms and study their sensitivity to antibiotics, as has been suggested by Ledbetter and Kutscher (1969). Nevertheless, our data suggest that many snakebite victims could be treated with penicillin plus a broad-spectrum antibiotic to suppress infection. In this regard, it should be noted that many clostridial strains are naturally resistant to clindamycin, an antibiotic recommended for use against anaerobic bacteria.

Brunker and Fernández (1974) and Ledbetter and Kutscher (1969) have reported that *Cl. bifermentans* is the spore-forming anaerobe most commonly isolated from snake venoms and mouth cavities. However, our data indicate that a much larger range of clostridial species are present and that they have an incidence approaching that of *Cl. bifermentans.* A likely explanation for this disparity is our use of more sensitive isolation techniques. This fact is significant, because

some of the other species found have been implicated in human infections (Balows et al., 1975).

Finally, it is important to point out that the sophisticated isolation techniques employed in this study to detect fastidious anaerobes failed to detect any but clostridial species in the mouth swabs and venoms tested. This would seem to greatly reduce the chances that anaerobes belonging to other genuses play a significant part in secondary infections of snakebite victims produced by the venom's bacterial flora.

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SUMMARY

The study reported here examined the aerobic and anaerobic bacterial flora of the mouth cavities and venoms of three Costa Rican snake species. Nearly all the venom samples contained bacteria, suggesting that secondary bacterial infection following snakebite is a real possibility.

Both aerobic and anaerobic organisms were isolated, fresh venoms yielding colony counts of aerobic organisms as high as 1×10^6 per ml. All the anaerobes isolated belonged to the genus *Clostridium,* the most common species being *Cl. bifermentans, Cl. butylicum, Cl. glycolicum, Cl. novyi* (type A), *Cl. sporogenes,* and *Cl. cadaveris.*

Where secondary bacterial infection of a snakebite victim is suspected, we recommend that the antibiotic sensitivity of the isolated organisms be determined so that a suitable antibiotic can be chosen. However, our results suggest that in an emergency, penicillin combined with a broad-spectrum antibiotic such as chloramphenicol or tetracycline may prove effective in most cases.

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MEASLES VACCINATION IN CANADA

Measles is a serious viral disease. Often regarded as being of little consequence, the illness can have severe and sometimes fatal complications. In Canada, about one in every 15 measles patients suffers from pneumonia or otitis media, about one in every 1,000 develops encephalitis, about one in every 10,000 dies.

An effective measles vaccine was introduced into Canada in 1963, and its widespread use has reduced the incidence of the disease in that country by approximately 90 per cent. Nevertheless, about 10,000 cases have been reported annually in Canada in recent years; this is presumably only a fraction of the actual total because many cases go unreported; and the data for 1979 show a marked rise in the incidence of measles in several provinces.

As of the end of 1979, a total of 22,257 cases (about 250 per 100,000 inhabitants under age 20) had been reported, an almost four-fold increase over the cases reported in 1978. Some outbreaks reported toward the end of the year also spread to other provinces in early 1980. The provinces most heavily affected in the first half of 1979 were Alberta, British Colombia, and Ontario, while during the period of October 1979 through January 1980 outbreaks were reported in Saskatchewan, Prince Edward Island, and Newfoundland.

In contrast, only 12,353 cases were reported in the United States during the first 41 weeks of 1979 (a rate of 18.5 per 100,000 inhabitants under age 18). This rate, the lowest ever recorded in the U.S., was 50 per cent below the rate in the corresponding period of 1978 and 75 per cent below the 1977 figure.

It is currently felt that legislation requiring vaccination of children in Canada at or before school entrance may be needed before the high levels of immunization needed to interrupt transmission and eliminate indigenous measles can be attained. Such legislation has been passed in all 50 states of the United States, and enforcement of that legislation has been instrumental in raising immunization levels and reducing the incidence of the disease.

Source: WHO, *Weekly Epidemiological Record* 55(40):305-306, 1980.