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The Aerobic and Anaerobic Flora of Rattlesnake Fangs and Venom

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Original Articles

The Aerobic and Anaerobic Flora of Rattlesnake Fangs and Venom

Therapeutic Implications

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Venom from 100 rattlesnakes and swabs of the fangs of 50 of these snakes were cultured for aerobic and anaerobic bacteria. Agar-dilution antibiotic susceptibilities for 170 of 207 aerobes and disc susceptibilities for 65 of 113 clostridia were determined. Clostridia were isolated from 48% of venom and 86% of fang specimens. Histotoxic species were found in 50% of the snakes. Ninety-seven strains of gram-positive cocci were isolated, but no coagulase-positive staphylococci. Of 110 strains of gram-negative rods, *Aerobacter, Proteus,* and *Pseudomonas* genera were most common. Four salmonella strains were found. All rattlesnake bites are potentially contaminated with clostridia and a wide variety of aerobic bacteria. Inflammation and necrosis secondary to envenomation provide a favorable setting for proliferation of organisms. The antibiotic susceptibility results reported here provide guidelines for selection of antibacterial therapy.

INFECTION complicating snakebite may be difficult to differentiate from inflammatory changes caused by envenomation itself.

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Although most deaths due to snakebite are primarily related to the size and kind of the snake involved[1,2] and failure to receive adequate amounts of antivenin,[3] infection influences morbidity and mortality.

Several sources of infection are possible. These include the snake's fangs and venom, the victim's skin and clothing, materials used for first aid treatment, and the hospital environment.

Reports concerning the bacterial flora of the oropharynx and venom of poisonous snakes are sparse and conflicting, and no data were found regarding antibiotic sensitivities of organisms isolated. Therefore, we collected venom from 100 rattlesnakes and swabbed the fangs of 50 of these snakes for aerobic and anaerobic culture and performed antibiotic susceptibility testing. The results of these studies can provide guidelines for the treatment of snakebite.

Materials and Methods

Western diamondback rattlesnakes (*Crotalus atrox*) were cultured. Many of the snakes cultured had been forced from hibernation by the spraying of gasoline into their dens. Specimens were collected within 48 hours after the snakes were removed from their natural habitat. All snakes cultured were active and appeared healthy with no evidence of injury or infection of the mouth. Venom and swabs of the fangs of 50 snakes were collected in March 1967; venom was collected from 50 additional snakes in February 1968.

Each snake's mouth was held open by a professional handler. The fang sheaths, which envelop the fangs except during a strike, were retracted and the fangs and their sheaths swabbed with two sterile cotton-tipped applicator sticks. The cotton-tipped portions of the applicator sticks were then broken off into sterile screw-capped tubes and immediately placed on dry ice. Venom was collected by placing each snake's fangs over the edge of a sterile 30-ml jar into which the venom was "milked" (Fig 1). Venom specimens collected in 1967 were placed on dry ice for transportation to Dallas, Texas, where they were stored at −70 C until cultured.

In 1968, venom specimens collected were not frozen, but were promptly transferred from each collecting jar with a sterile pipette to tubes of cooked-meat medium. These venom cultures were kept at ambient temperature for 24 hours and were then incubated at 37 C for 48 hours.

Ten of the venom and fang specimens collected in 1967 were thawed, divided equally, and transferred to 20-ml tubes containing 10 ml of thioglycollate broth medium and 1 ml of ascitic fluid. Duplicate specimens were incubated at room temperature or at 37 C for 48 hours. The remaining venom specimens and fang swabs collected in 1967 were thawed and transferred to cooked-meat medium and incubated at 37 C for 48 hours.

Subsequent to the culture procedures described above, all remaining specimens collected in 1967 and those collected in 1968 were subcultured to blood agar, eosin methylene-blue agar, and blood agar base medium containing azide for aerobic incubation at 37 C for 24 hours. For

Table 1.—*Results of Cultures From 100 Rattlesnakes*

Specimens		% of Specimens		
Source	No.	Aerobic Growth	Anaerobic Growth	No Growth
Fangs (1967)*	50	94	86	6
Venom (1967)*	50	32	24	50
Venom (1968)†	50	84	72	10

*Specimens frozen before primary culture inoculation.
†Specimens inoculated directly to culture medium without prior freezing.

Table 2.—*Aerobic Gram-Positive Cocci Isolated From 100 Rattlesnakes*

	Source		
Organism	Fangs (1967)	Venom (1967)	Venom (1968)
Enterococci	26	0	21
Viridans streptococci	2	1	17
Micrococci (coagulase negative)	20	10	0
Total	48	11	38

Table 3.—*Aerobic Gram-Negative Rods Isolated From 100 Rattlesnakes*

	Source		
Organism	Fangs (1967)	Venom (1967)	Venom (1968)
Aerobacter genus	10	1	12
Alkaligenes fecalis	1	0	0
Arizona genus	2	0	0
Bethesda-Ballerup	0	0	1
Citrobacter	10	0	2
Enterobacter hafnia	0	0	6
Escherichia coli	4	0	3
Herellea genus	1	0	1
Klebsiella pneumoniae	1	0	3
Proteus genus	4	5	6
Pseudomonas genus	11	0	0
Salmonella genus	1	1	2
Serratia genus	7	1	4
Unidentified	7	3	0
Total	59	11	40

Table 4.—*Clostridial Species Isolated From 100 Rattlesnakes*

	Source		
Clostridium Species	Fangs (1967)	Venom (1967)	Venom (1968)
C aerofoetidum	0	2	0
C bifermentans*	14	2	11
C capitovale	0	0	1
C feseri	3	0	2
C innocuum	0	1	0
C novyi A*	0	0	1
C paraputrificum	1	0	1
C perfringens*	6	0	12
C septicum*	7	0	3
C sordellii*	0	0	8
C sporogenes	3	3	0
C subterminale	1	0	1
Species unidentified	18	4	8
Total	53	12	48

*Species histotoxic to man.

anaerobic studies, each specimen was subcultured to blood agar and to blood agar base medium containing yeast extract (Difco), menadione (0.2 mg/liter), hemin (5.0 mg/liter), and neomycin sulfate (50 mg/ml) and incubated in Brewer jars at 37 C for 24 to 48 hours. Additional subcultures to cooked-meat medium were incubated for three weeks to provide an adequate incubation period for bacteroides organisms.

Aerobic organisms were identified by standard bacteriologic, biochemical, and serologic methods.

Anaerobic, catalase-negative, gram-positive rods isolated were presumptively considered to be clostridia and were subcultured to blood agar plates for aerobic and anaerobic incubation, to 10 ml tubes containing 8 ml of freshly bottled thioglycollate medium and 0.5 ml of horse serum (Difco), to cooked-meat dextrose medium, and to egg-yolk agar for anaerobic incubation. Specific identification of clostridia was done according to the methods of the Laboratory Consultation and Development Branch of the National Communicable Disease Center, Atlanta.[4] Specific characteristics determined for each clostridial strain included colony morphology, hemolysis, motility, and the position, configuration, and location of spores. Each organism was tested for the production of urease, gelatinase, indole, hydrogen sulfide, acid, and gas. Carbohydrate fermentation tests included glucose, mannitol, lactose, sucrose, maltose, salicin, glycerol, and starch. Each clostridial strain was tested for lecithinase and lipase activity, nitrate reduction, and digestion of milk, meat, and serum.

Plate-dilution antibiotic susceptibilities were determined for 170 strains of aerobic organisms isolated from the fang and venom specimens. Antibiotic disc-diffusion susceptibilities were

Fig 1.—Method of collecting venom specimens.

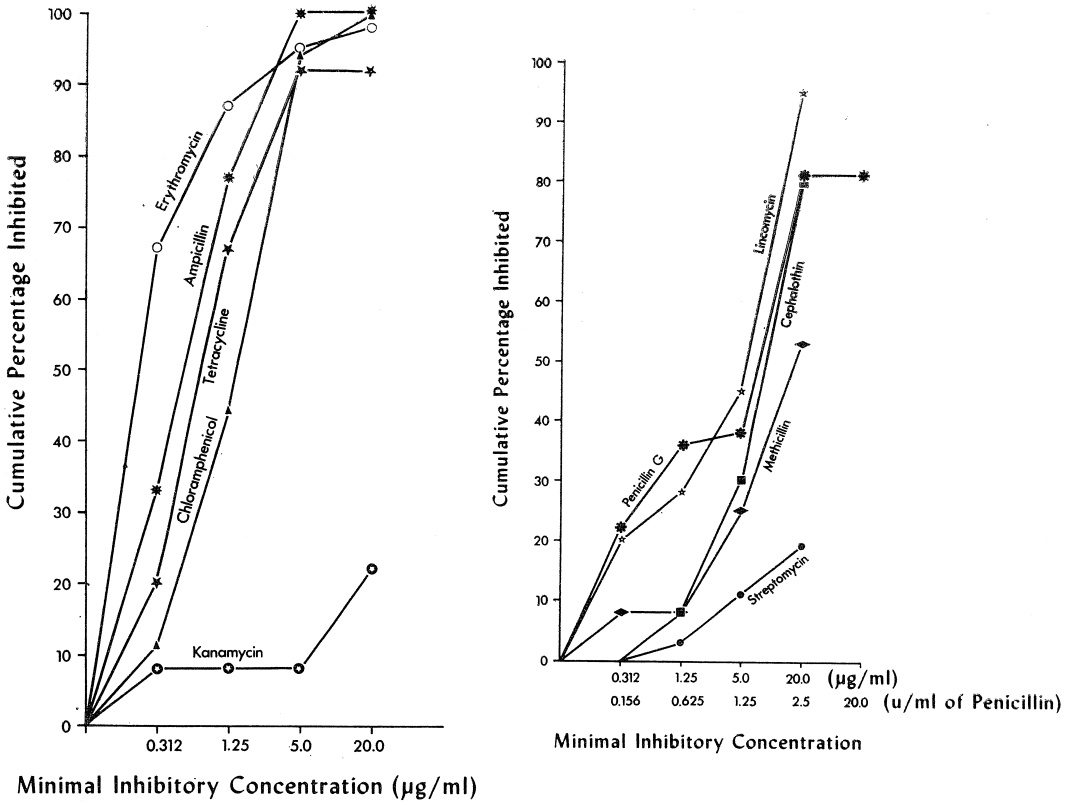

Fig 2.—Antibiotic susceptibilities of 36 strains of aerobic grampositive cocci isolated from rattlesnake venom (by agar dilution method).

determined for 65 clostridial strains by the following method. Two 10 ml-tubes, each containing 8 ml of brain-heart infusion agar, were heated to melting and cooled to 50 C. One milliliter of an actively growing 18-hour broth culture of the organism to be tested was added to each of the two tubes of infusion agar. After thorough mixing, the contents of both tubes were poured into a sterile Petri dish and allowed to solidify. Antibiotic sensitivity discs were then applied to the surface of the agar and the plates were incubated upright in a Brewer jar at 37 C for 48 hours. Zones of inhibition were read against a black background.

Results

The aerobic and anaerobic culture results are summarized in Table 1. The lower yield of both aerobic and anaerobic organisms obtained from the venom cultures in 1967, as compared with the 1968 fresh wet venom specimens, is most likely attributable to the initial freezing and drying of the venom in the 1967 procedure. Also, there was a lower yield of anaerobes from the venom and fang specimens transferred to thioglycollate broth containing ascitic fluid than from cooked-meat media cultures. Incubation of these specimens at room temperature or at 37 C did not significantly influence the yield of aerobic or anaerobic organisms.

Ninety-seven strains of aerobic, gram-positive cocci were isolated (Table 2). Again, a lower yield was noted from the venoms collected in 1967 as compared to those cultured in 1968. No coagulase-positive staphylococci were found.

One hundred ten strains of gram-negative rods were found (Table 3). *Aerobacter, Proteus* and *Pseudomonas* were isolated most frequently. Four salmonelleae strains were isolated, including three from venom. Two of these were *Salmonella newport;* serotyping was not done on the other two.

Clostridia were the only anaerobes cultured from fangs or venom. Species identi-

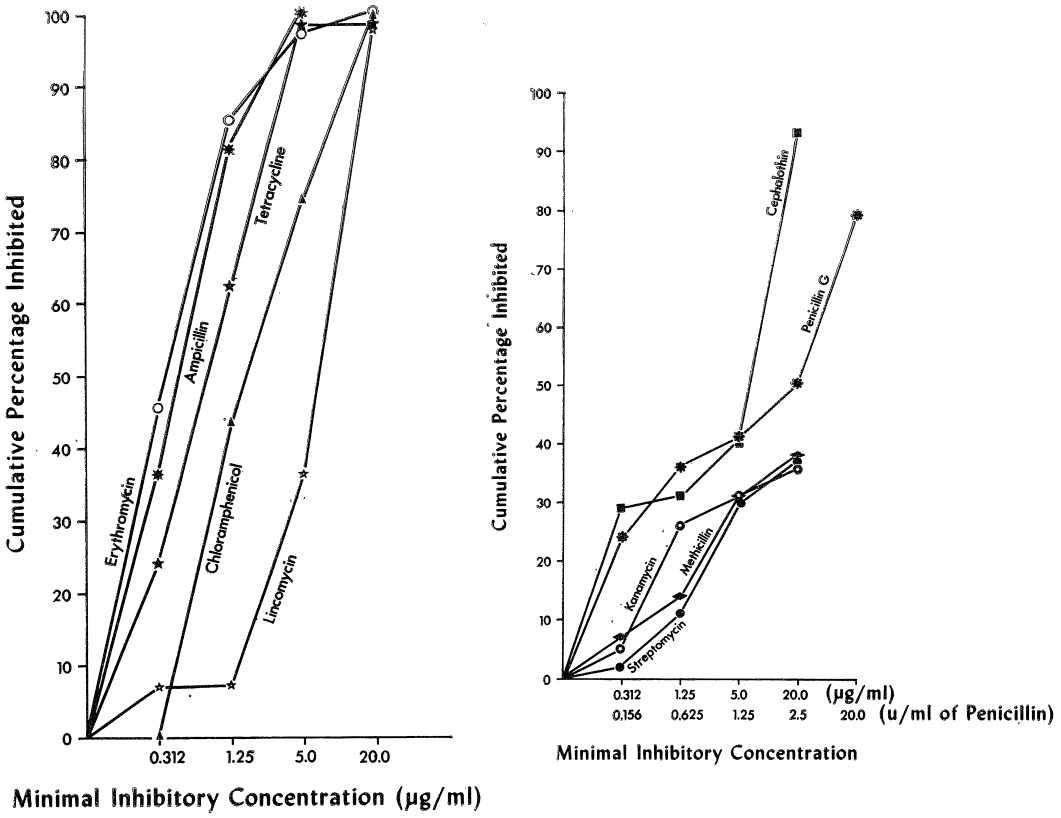

Fig 3.—Antibiotic susceptibilities of 42 strains of aerobic gram-positive cocci isolated from rattlesnake fangs (by agar dilution method).

fication was accomplished for 83 of 113 strains of clostridia cultures (Table 4). Sixty-four were histotoxic species, that is, capable of producing gas gangrene; these histotoxic species were from 50 of the snakes studied. No *Clostridium tetani* were identified. Three different species of clostridia were isolated from each of two fang cultures and from each of two venom specimens. Two clostridial species were isolated from each of seven fang cultures and from each of seven venom specimens. While the method of venom collection used could have allowed contamination of the venom with mouth organisms, most clostridia isolated from the venom specimens were different species from those isolated from the same snake's fangs.

Results of the antibiotic susceptibility testing of 65 clostridia are as follows:

65 strains tested
  All inhibited by:
    Penicillin G potassium (10μ)
    Erythromycin (15μg)
    Tetracycline (30μg)
    Chloramphenicol (30μg)
  61/65 inhibited by:
    Lincomycin hydrochloride monohydrate (2μg)
48 strains tested
  All inhibited by:
    Ampicillin (10μg)
    Cephalothin sodium (30μg)

Forty-four of the clostridia tested were histotoxic species. Fourteen were *C perfringens*, the clostridial species most often associated with clinical gas gangrene. Four of the 65 clostridial strains were not inhibited by a 2-μg disc of lincomycin hydrochloride monohydrate and a very narrow zone of inhibition was present with many other strains. All strains were sensitive to the other antibiotics tested at the disc concentrations used. The 48 strains tested were inhibited by ampicillin and cephalothin sodium.

Most of the aerobic gram-positive cocci

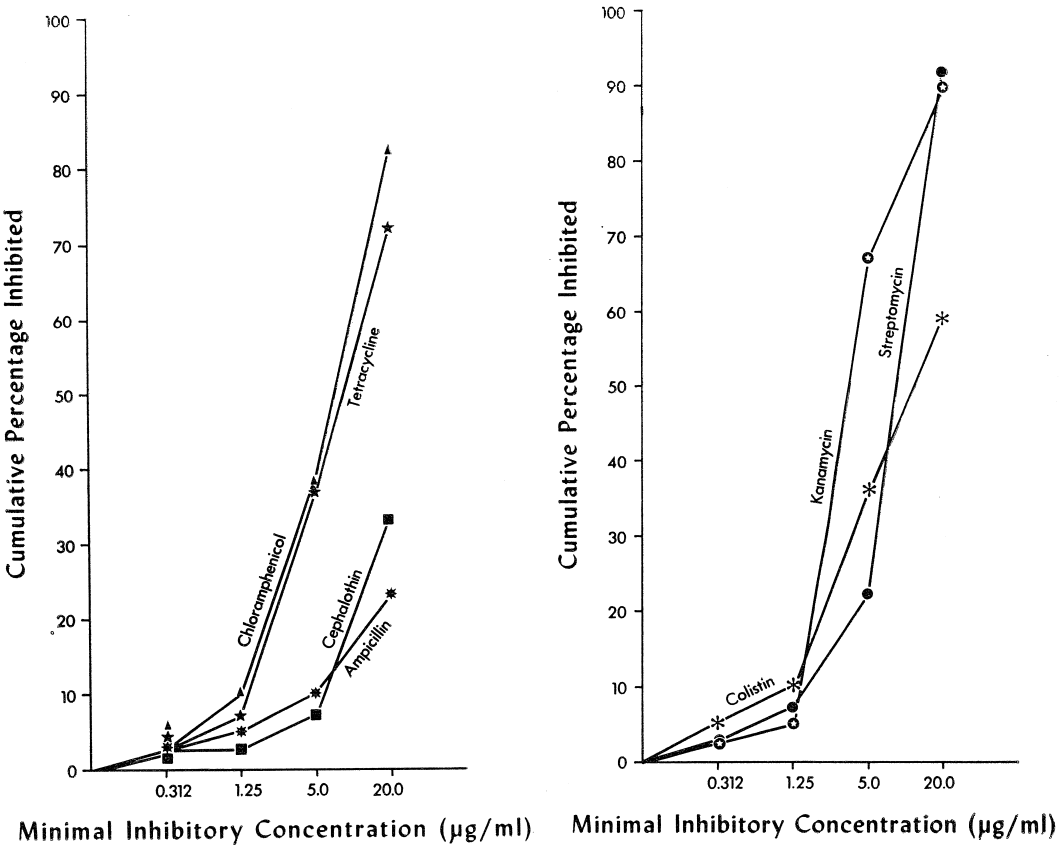

Fig 4.—Antibiotic susceptibilities of 39 strains of aerobic gram-negative rods isolated from rattlesnake venom (by agar dilution method).

from venom were susceptible to erythromycin, ampicillin, tetracycline, and chloramphenicol (Fig 2, *left*). Sixty-two percent of these organisms required greater than 1.25 units/ml of penicillin G potassium for their inhibition, while methicillin sodium cephalothin sodium, and streptomycin sulfate were even less effective (Fig 2, *right*). The antibiotic susceptibility testing of the gram-positive cocci isolated from fangs gave similar results (Fig 3).

Most of the gram-negative rods isolated from venom and fangs were resistant to the usual therapeutically attainable concentrations of all antibiotics tested (Fig 4 and 5). While kanamycin sulfate, tetracycline, and chloramphenicol appeared to be more effective than the other antibiotics used, many strains were resistant to kanamycin sulfate and fewer than 50% were susceptible to tetracycline, chloramphenicol, and streptomycin sulfate at the level of 5μg/ml.

Comment

Different culture methods used may account for the variation in results of other investigators' attempts to determine the bacterial flora of the oropharynx and venom of poisonous snakes, especially the failure of some investigators to isolate clostridia.[5,6]

Jackson found *C perfringens* in the mouths of all 51 snakes he cultured.[7] However, his methods of culture were not described.

Using alkaline-meat medium, Williams et al[8] recovered clostridia from the mouths of all 49 Australian snakes cultured, but costridia were isolated from only five of 50 wet venom specimens and no anaerobes were found in 50 dry venom specimens. Coliform organisms and gram-positive cocci were also isolated from most of the snakes' mouths.

Parrish et al[5] used thioglycollate medium, MacConkey's agar, brain-heart infusion

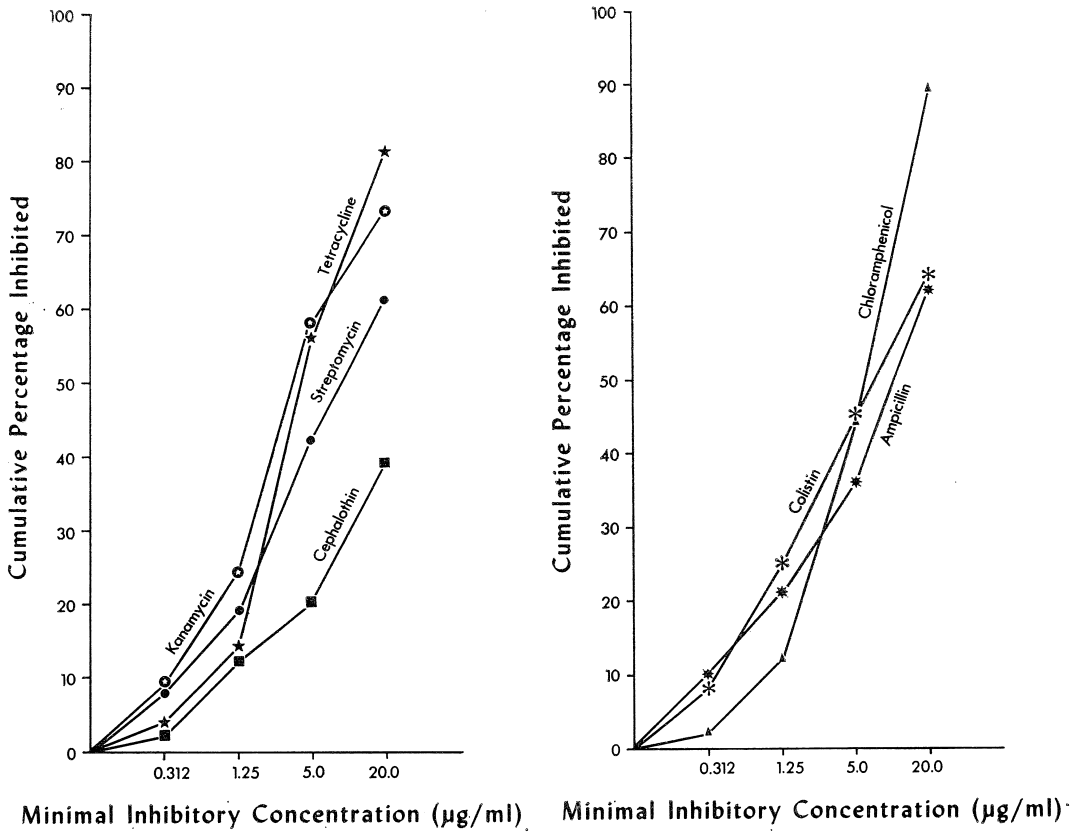

Fig 5.—Antibiotic susceptibilities of 53 strains of aerobic gram-negative rods isolated from rattlesnake fangs (by agar dilution method).

broth, and blood agar plates to culture the oropharynx and venom of 25 North American pit vipers. Twenty-eight percent of the venom cultures were sterile and no clostridia were isolated from venom. Thioglycollate broth is not an ideal medium for the isolation of clostridia. All of the oropharyngeal cultures were positive for various aerobic gram-negative rods or gram-positive cocci or both, but clostridia were recovered from only one specimen.

Fischer et al[6] diluted the mouth swabbings and venom specimens obtained from each of 45 North American pit vipers with 5 ml of 1% peptone broth before transferring a loop of this material to a variety of culture media. Many aerobic organisms were isolated, but despite the use of recently boiled thioglycollate medium no anaerobes were recovered. Cooked-meat medium was not used.

We found only two clostridia in ten frozen venom specimens which had been thawed and transferred to thioglycollate broth containing ascitic fluid. For this reason the remaining venom specimens were transferred to cooked-meat medium and a higher yield of clostridia was obtained.

Our culture methods should have been adequate for the isolation of bacteroides. The failure to find them in any specimen suggests that these organisms are not part of the oral flora of rattlesnakes. We found no reports concerning the isolation of bacteroides from snakes. These organisms do inhabit the oropharynx of man and could conceivably be introduced at a bite site when oral suction is used as a first aid measure.

Other investigators reported the isolation of a few strains of *Staphylococcus aureus;* however, coagulase testing was not done.[5,8] We failed to isolate coagulase-positive staphylococci from any specimen. Should staphylococcal infection complicate snake-

bite, we suggest it be regarded as hospital-acquired for treatment purposes until antibiotic sensititives are performed.

We were unable to find previous reports of the isolation of salmonelleae from the oropharynx or venom of poisonous snakes. In view of the widespread distribution of *Salmonella* genus in nature, it was not surprising that we encountered them in four specimens.

The incidence of infection complicating snakebite is unknown, but is thought to be very low, especially in the absence of severe tissue necrosis.[9] There is pharmacologic similarity between the enzymes of pit viper venom and the toxins produced by clostridia.[10,11] Therefore, systemic and local tissue changes produced by envenomation and by clostridial infection can be so similar as to make clinical differentiation virtually impossible. When infection is suspected, needle aspiration of the involved area is recommended to obtain material for smears and bacterial stains and for aerobic and anaerobic cultures.

Contamination of wounds with *C perfringens* and other clostridial species is common, but significant infection is rare.[12] Although crepitant cellulitis is characteristic of clostridial infection, other anaerobic as well as some aerobic organisms can produce gas,[13] emphasizing the importance of specific bacteriologic confirmation of the etiology of an infection. Should gas gangrene be suspected, surgical exploration of the wound with inspection of the muscle and appropriate debridement are indicated. Antibiotics and gas gangrene antitoxin are advised for the treatment of gas gangrene. The prophylactic value of gas gangrene antitoxin is uncertain.[14]

Only a few cases of proved clostridial infection complicating snakebite were found in the English literature. MacLennan's review of the histotoxic clostridial infections of man contains none related to snakebite.[11] The extensive review of anaerobic infections by Bornstein et al does not mention any association between snakebite and anaerobic infection.[12] A personal communication from Newton C. McCollough, MD, dated April 22, 1968, states that his investigations in the southeastern states led to the documentation of one fatal case of clostridial infection (type unknown) treated with high femoral amputation. He also observed a well documented case of tetanus subsequent to snakebite.

In a review of 47 cases of unusual wounds associated with tetanus in the United States during 1965 and 1966, none was associated with snakebite.[15] A letter from Lowell S. Young, MD, dated April 8, 1968, states that no case of tetanus associated with snakebite has been recorded during the three-year existence of the tetanus surveillance system of the National Communicable Disease Center.

In 1908, Willson[16] presented a second-hand report of a case of tetanus with onset one week after a rattlesnake bite on the thumb. The patient had received no prior medical attention and died within two days. Evans and Farrell[17] cite a personal communication as the only reference to tetanus subsequent to snakebite.

Parrish[18] reported a documented case of tetanus following a cotton-mouth moccasin bite. There was extensive necrosis of the victim's left lower leg. The patient had never received tetanus immunizations. In spite of initial treatment with excision of the wound, tetanus antitoxin, and penicillin, tetanus occurred six days after the bite and the patient died 48 hours later.

No *C tetani* were identified in the specimens we studied and no report of the isolation of *C tetani* from snakes was found. Nevertheless, tetanus prophylaxis should be provided for any puncture wound since spores may be present on the patient's skin or clothing.

The potentially histotoxic clostridia are so commonly present in the venom and oropharynx of rattlesnakes that all bites with extensive necrosis should be treated with antibiotics effective against clostridia. The clostridia tested in this study were sensitive to several antibiotics. Recently, *C welchii* resistant to 5μg of tetracycline have been reported.[19] Penicillin G potassium is considered the best drug for the treatment and prophylaxis of clostridial infection; erythromycin and chloramphenicol are also effective.[19]

None of the antibiotics tested was effective against the majority of gram-negative organisms. Attempted prophylaxis of all

snakebite patients with broad-spectrum or multiple antibiotics, hoping to eradicate all organisms present, is clearly not feasible. Our data show that penicillin and streptomycin sulfate, the antibiotic combination most commonly employed, would be a poor choice on a statistical basis. Antibiotic therapy for infected wounds should be based on cultures and antibiotic susceptibility testing.

Optimal methods for the management of the patient with pit viper envenomation, including local wound management and antivenin administration, are summarized in a recent article by the Subcommittee on Venomous Snakebite under the Board of Governors of the Florida Medical Association.[20]

Our data indicate that all rattlesnake bites are potentially contaminated with clostridia as well as a wide variety of aerobic bacteria. In contrast with the gradual production of clostridial toxins, the enzymes present in venom have a capacity to begin tissue destruction immediately providing an ideal setting for proliferation of organisms present.

The risk of infection is minimized by neutralization of the venom with antivenin and by avoiding methods of treatment that impair circulation, enhance tissue necrosis, and reduce the oxidation-reduction potential of the tissues.

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Western diamondback rattlesnakes were provided by the Sweetwater Jaycees, Sweetwater, Tex.

Generic and Trade Names of Drugs

Erythromycin—*Erythrocin, Erythrogran, Erythroguent, Ilotycin.*

Tetracycline—*Achromycin, Panmycin, Tetracyn, Steclin, Rexamycin.*

Chloramphenicol—*Chloromycetin.*

Lincomycin hydrochloride monohydrate—*Lincocin.*

Ampicillin—*Omnipen, Penbritin, Polycillin, Principen.*

Cephalothin sodium—*Keflin.*

Methicillin sodium—*Dimocillin-RT, Staphcillin.*

Kanamycin sulfate—*Kantrex.*

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