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Article *in* The Journal of Infectious Diseases · December 1979

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THE JOURNAL OF INFECTIOUS DISEASES • VOL. 140, NO. 5 • NOVEMBER 1979
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Bacteriology of Rattlesnake Venom and Implications for Therapy

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Although the incidence of infection secondary to the bites of venomous snakes remains unknown, the routine use of prophylactic antimicrobial therapy is advocated. In this study, the venom from 15 rattlesnakes was cultured, and 58 aerobic and 28 anaerobic strains of bacteria were isolated. The most common species isolated were *Pseudomonas aeruginosa, Proteus* species, coagulase-negative staphylococci, and *Clostridium* species. *Bacteroides fragilis* was also recovered. When the fang sheaths of four additional rattlesnakes were retracted and the fangs of these snakes decontaminated, 50% of the samples of venom had no bacterial growth ($P = 0.035$). Until a clinical study is performed, the use of antimicrobial therapy that reflects the complex oral flora of rattlesnakes is still recommended in most cases of envenomization.

Envenomization or poisoning from snake venom is a significant worldwide health problem and causes an estimated 40,000–50,000 deaths each year [1]. In the United States approximately 8,000 persons are bitten by venomous snakes yearly [2, 3]. Although these wounds have been characterized as "contaminated, venom-laden, anaerobic puncture wounds, which predispose to infection and tissue destruction" [1] and although the use of antimicrobial therapy is advocated [1, 3–8], the exact role of infection—and even the incidence of infection—remain unknown.

The initial stage in an attempt to establish what, if any, constitutes proper antimicrobial therapy requires that the bacteriology of rattlesnake venom be determined. Previous reports on the bacterial flora of the oropharynx and venom of poisonous snakes are sparse and conflicting [1, 8–11]. Therefore, we performed both aerobic and anaerobic cultures on the venom of 19 rattlesnakes.

Received for publication April 5, 1979, and in revised form July 6, 1979.

This study was supported in part by the Veterans Administration Medical and Research Services and by grant no. 2-R01-NS-11476-Tox from the National Institutes of Health.

This paper was presented, in part, at the annual meeting of the American Federation for Clinical Research, Western Section, Carmel, California, February 9, 1979, and at the annual meeting of the American Society of Microbiology, Honolulu, Hawaii, May 9, 1979.

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Materials and Methods

Collection of venom. Venom from nine *Crotalus viridis helleri* (Southern Pacific rattlesnake) and six *Crotalus scutulatus scutulatus* (Mojave rattlesnake) was collected. The snakes were all healthy. The amount of time that they had been in laboratory captivity varied from two weeks to two and one-half years. Snakes were fed either live mice or rats. No killed meat products, antibiotics, or antiseptics were used in feeding. No snake had been milked within three weeks before the experiment was conducted. To collect the venom we placed each snake's fangs through Parafilm M® (Marathon Products, Greenwich, Conn.) over a sterile jar. We did not retract the fang sheaths. Venom was immediately transferred with a sterile pipette and inoculated onto the culture media.

Samples of venom from four *Crotalus atrox* (Western diamondback rattlesnake) were obtained in the same manner, except that the fang sheaths were retracted and the fangs were swabbed with alcohol before the venom was collected.

Preparation of cultures. Specimens of venom were inoculated immediately after collection onto the following media: MacConkey's agar; brucella blood agar supplemented with vitamin K_1 (10 μg/ml), hemin (5 μg/ml), and 5% sheep blood (BAP); and phenylethyl alcohol blood agar (PEA), and

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incubated aerobically at 35 C. BAP, PEA, and kanamycin-vancomycin laked blood agar that was supplemented with vitamin K_1 and hemin (all of these media had been reduced for at least 48 hr in an anaerobic chamber before use) were incubated anaerobically at 35 C in GasPak® jars (Baltimore Biological Laboratories, Cockeysville, Md.) after inoculation.

Specimens were processed, and anaerobic isolates were identified by the procedures outlined in the *Wadsworth Anaerobic Bacteriology Manual* [12] and the *Anaerobe Laboratory Manual* [13]. Aerobic and facultative aerobic bacteria were identified by standard criteria. [14].

Results

When the fang sheaths were not retracted and the fangs were not decontaminated before collection of venom, 58 strains of aerobic (table 1) and 28 strains of anaerobic (table 2) bacteria were isolated. *Proteus* species (10 strains), *Pseudomonas aeruginosa* (nine strains), and coagulase-negative staphylococci (10 strains, six of which were novobiocin-resistant) were the most common aerobic bacteria isolated. Only two strains of *Streptococcus* and one strain of *Staphylococcus aureus* were recovered. One isolate of *Arizona hinshawii (Salmonella arizonae)* was recovered. The most commonly recovered anaerobic bacteria were *Clostridium* species (22 strains), including six strains of *Clostridium cadaveris*, three of *Clostridium perfringens*, three of *Clostridium sordellii*, and two of *Clostridium septicum*. In addition, *Bacteroides fragilis* was isolated from five of the 15 specimens. Most of these strains were recovered in heavy growth.

When the fang sheaths were retracted and the fangs were swabbed with alcohol before collection of venom, two samples had no aerobic or anaerobic bacteria isolated (Fisher's exact probability test, $P = 0.035$) and one sample had only three strains of aerobic bacteria isolated (one colony each of *P. aeruginosa, Alcaligenes* species, and *Pseudomonas maltophilia*). A fourth sample had six aerobic and five anaerobic strains of bacteria isolated in very light growth. The bacteria isolated were *Alcaligenes* species, *Proteus mirabilis, P. maltophilia, Micrococcus* species, *Corynebacterium* species, coagulase-negative staphylococci (novobiocin-resistant strains), *C. perfringens, C. cadaveris, Clostridium bifermentans*, and *Clostridium limosum*.

Table 1. Species of aerobic and facultative aerobic bacteria isolated from the venom of rattlesnakes.

Species	Source of isolate: *Crotalus viridis helleri*	Source of isolate: *Crotalus scutulatus scutulatus*
Acinetobacter species	1	0
Alcaligenes species	4	2
Bacillus species	2	4
Citrobacter species	1	0
Corynebacterium species	1	1
Enterobacter cloacae	4	0
Micrococcus species	2	1
Proteus mirabilis	2	1
Proteus morganii	2	2
Proteus rettgeri	1	3
Pseudomonas aeruginosa	8	1
Pseudomonas maltophilia	1	0
Arizona hinshawii	1	0
Staphylococcus aureus	0	1
Staphylococcus species (coagulase-negative)*	7	3
Streptococci, α-hemolytic	0	1
Streptococci, β-hemolytic (group B)	1	0
Total	38	20

NOTE. The fang sheaths of the rattlesnakes were not retracted, and the fangs were not decontaminated before the samples of venom were collected.

* Includes six novobiocin-resistant strains, three from each source.

Table 2. Species of anaerobic bacteria isolated from the venom of rattlesnakes.

Species	Source of isolate: *Crotalus viridis helleri*	Source of isolate: *Crotalus scutulatus scutulatus*
Bacteroides fragilis	4	1
Clostridium species	4	2
Clostridium cadaveris	4	2
Clostridium carnis	1	0
Clostridium paraputrificum	0	1
Clostridium perfringens	2	1
Clostridium septicum	2	0
Clostridium sordellii	1	2
Propionibacterium acnes	1	0
Total	19	9

NOTE. The fang sheaths of the rattlesnakes were not retracted, and the fangs were not decontaminated before the samples of venom were collected.

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Before this study, we had cultured the oropharynx of another *C. viridis helleri* and had isolated four strains of aerobic and 14 strains of anaerobic bacteria, including six *Clostridium* species. We also had cultured three uninfected snakebite wounds by previously described techniques [15] and had had only light growth of *Propionibacterium acnes*, a probable contaminant, from one wound.

Discussion

The routine use of empiric antimicrobial therapy in cases of envenomization is frequently advocated [1, 3–8]. This approach has been based upon the sparse and often conflicting reports of the normal flora of snake venom [1, 2, 8, 10, 11], since the incidence of infection and wound contamination have not been studied. Previous reports have noted the recovery of a variety of aerobic and anaerobic bacteria from samples of rattlesnake venom. However, attempts to avoid gross contamination of the venom with the flora of the saliva, fang sheaths, or fangs had not been attempted. "Naturally, venom collecting as a drop on the tip of the snake's non-sterile fang can not hope to escape occasional contamination" [11]. We attempted to avoid such contamination in four of the snakes by first swabbing the fangs with alcohol and then keeping the fang sheaths retracted during collection of venom. Although the numbers are small, our results show that 50% of such specimens had no bacterial growth and that the other 50% had very light bacterial growth ($P = 0.035$, Fisher's exact probability test). This finding suggests that previous reports of bacteria in rattlesnake venom reflect the oral flora of the rattlesnake rather than the flora of venom itself. It seems probable that the oral flora of the rattlesnake, introduced in the victim at the time of injury, may be responsible for secondary infection and that rattlesnake venom, like other body fluids, is sterile. Previous investigators had noted that 10%–30% of potentially contaminated venom specimens were sterile [10, 11].

The question of whether snakes in captivity and wild snakes are microbiologically comparable remains unanswered. Some researchers [9, 11] have reported sparse bacterial growth from oropharyngeal and venom specimens of freshly caught snakes, as compared with "very numerous oral bacterial flora" [11] in captive snakes. However, others [1, 8] have noted the moderate to heavy growth of a wide variety of bacteria from specimens of venom from freshly caught snakes. Jackson [10] isolated *Clostridium welchii* (now *C. perfringens*) in the mouths of 50 captive and one wild snake and in 50% of the cultures that had been obtained from "the fresh wounds in snake bite patients." Although different methods of preparation of cultures may account for some variation, it is more probable that the variation of the fecal flora of the live prey that had been ingested by the snakes is responsible. As the snake consumes its prey (usually small rodents and some lizards in the wild), these animals frequently defecate. All of our captive snakes were fed live rodents, but those studies that noted a difference in flora between wild and captive snakes [9, 11] did not specify the food of their captive snakes. These researchers also noted that many of the mouths of the captive snakes were "suppurative" [9] or had "cancre" [11]; all of our rattlesnakes were healthy. Although there are probably differences in the fecal flora of laboratory and wild rodents, it is also probable that the flora of rodents in the wild varies according to each locale.

Our study supports the finding of Ledbetter and Kutscher [8], Jackson [10], and Williams et al. [11] that the *Clostridium* species are the most common anaerobic species of the oral flora. *B. fragilis* had not been previously reported to be present in the flora of rattlesnakes; we found *B. fragilis* in one-third of our specimens. The spectrum of our aerobic isolates is generally similar to that of other studies [1, 8–11], with *Pseudomonas* species and *Proteus* species being the most common isolates. However, we also found many novobiocin-resistant, coagulase-negative staphylococci, one strain each of *S. aureus* and *A. hinshawii*, and no enterococci in these specimens. This normal oral flora may contaminate the wounds of envenomized patients, but it is not possible to generalize from our experience with the three snakebite wounds that were cultured, especially since each patient had had several forms of therapy (antimicrobial therapy, debridement, washing of the wound) before we obtained our cultures.

In consideration of the devitalization and necrosis of tissue secondary to envenomization and the potential for contamination with the normal flora

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of the mouth of the rattlesnake, the use of prophylactic antimicrobial therapy for the treatment of venomous snakebite wounds seems warranted. The selection of a drug for empiric therapy should reflect the complex aerobic, facultative aerobic, and anaerobic flora of the snake oropharynx, until results from cultures are available. The current recommendation for tetanus prophylaxis also seems warranted. A clinical study, in which cultures are obtained before first aid therapy, is needed to determine definitively the risk and rate of infection.

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