Bacteriology of *Naja atra* Snakebite Wound and Its Implications for Antibiotic Therapy

Yan-Chiao Mao, Po-Yu Liu, Liao-Chun Chiang, and Chen-Chang Yang

1 Introduction

Six major venomous snake species are distributed throughout Taiwan, including *Trimeresurus stejnegeri*, *Protobothrops mucrosquamatus*, *Deinagkistrodon acutus*, and *Daboia siamensis* in the Viperidae family and *Naja atra* and *Bungarus multicinctus* in the Elapidae family. *N. atra*, the only cobra species, is also distributed throughout Southeastern Asia, including Vietnam, Laos, and Southern China (Fig. 1) [1]. In Taiwan, *N. atra* infrequently bites humans and causes 6% (range, 0%–36%) of all snakebite cases [2]. However, once envenomated, the majority of patients develop wound infections, including cellulitis, tissues necrosis, finger or toe gangrene, and/or extensive necrotizing fasciitis (Figs. 2 and 3); therefore, empirical antibiotic therapy is frequently advocated [3]. In Taiwan, bacteriology studies of *N. atra* bite wounds remain scarce and fragmented [4–6]. Although studies of the oral bacteriology of *N. atra* have been conducted in Hong Kong [7, 8], little is known about snakebite wound bacteriology and the effects of geographic differences in the same species [9–11]. To better understand the bacteriology of *N. atra* bite wounds, we retrospectively analyzed 112 cases from two referring medical centers: Taichung Veterans General Hospital (VGH-TC) in central Taiwan and Taipei Veterans General Hospital (VGH-TP) in Northern Taiwan.

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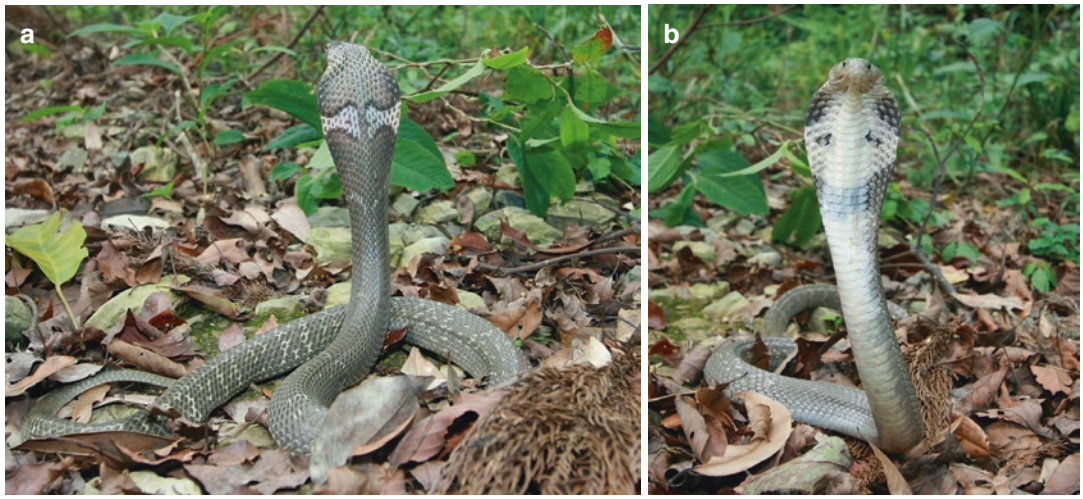

Fig. 1 Features of *Naja atra* (pictures were provided and used with the permission from Chih-Ming Lai). (**a**) *N. atra* (dorsal side). The hood mark shape is variable from spectacle, mask to horseshoe, or O-shape and is often linked to light throat area on at least one side. (**b**) *N. atra* (ventral side). The throat area is clearly defined light which is usually with a pair of clearly defined lateral spots

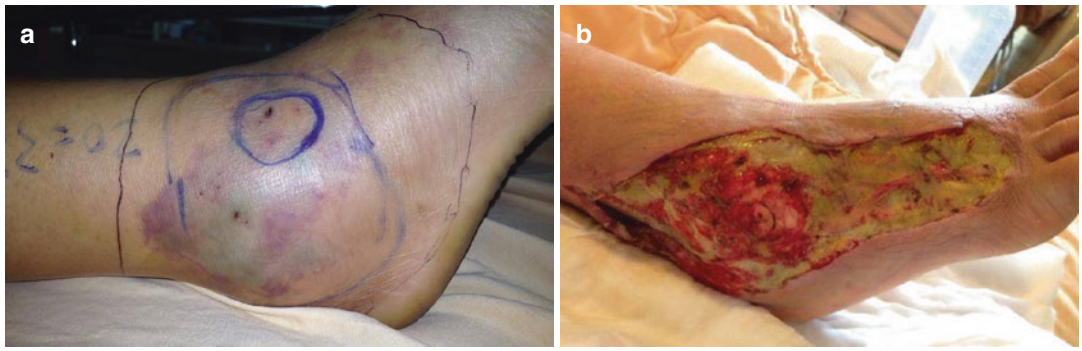

Fig. 2 (**a**) *N. atra* bite over right foot, manifesting local tissue necrosis and abscess formation which occurred 33 h later. (**b**) Second debridement was performed 7 days after the bite. *M. morganii* and *E. faecalis* were identified in the deep tissue biopsy culture

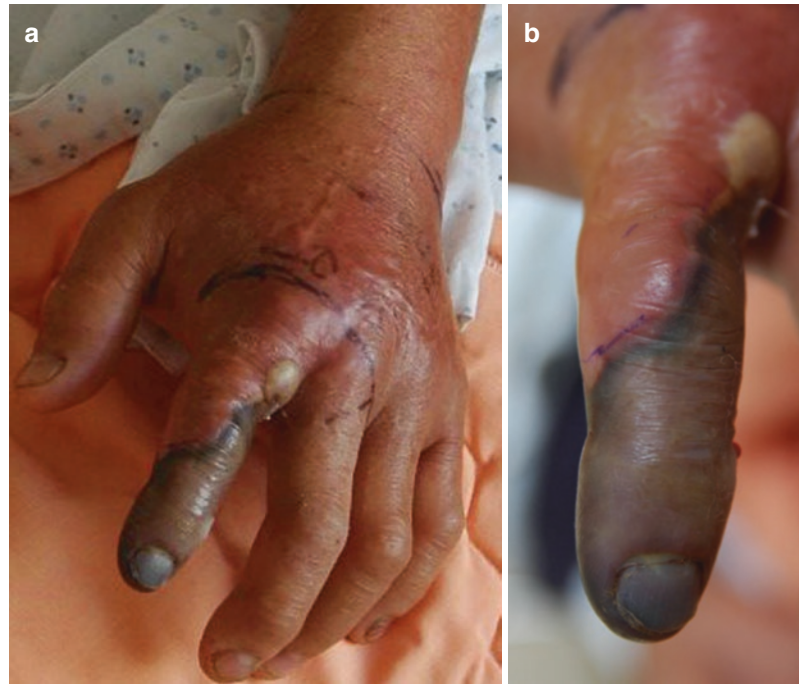

Fig. 3 (**a**) *N. atra* bite over left index finger. Swelling extended to the ipsilateral shoulder, and gangrenous change in the finger developed 3 days later. (**b**) A close-up picture. Patient underwent finger amputation 5 days post-bite. *A. hydrophila* was identified in the wound discharge culture

2 Study Population

This was a retrospective cohort study. The study protocol followed the principles of the Declaration of Helsinki. All cases of *N. atra* envenomation were admitted to VGH-TC between April 2005 and July 2009 (4 years) and to VGH-TP between October 1995 and September 2009 (14 years). Cases were identified by searching the computerized databases at both VGH-TC and VGH-TP, using the keywords "snake," "cobra," "*N. atra*," and "*N. n. atra*" both in English and Chinese. Two authors independently reviewed the medical records of all subjects with possible cobra envenomations. A definite diagnosis was made by the identification of the culprit snake, which included the examination of the snake, identification of the snake by the patient through a picture, or laboratory testing of the venom by the treating physician [12–14]. Patients with typical manifestations, as determined through physical examination, serial wound inspection, a relevant history, and clinical improvement after receiving specific antivenom for *N. atra*, were included in the "clinical case" group (Table 1) [2, 3, 15]. After a careful review of the medical records, patients with snakebites other than those of *N. atra* [e.g., patients with snakebites of the other five medi-

Table 1 Bacteria isolated from *Naja atra* bite cases and diagnostic methods of its envenomation

	Diagnostic methods							
	VGH-TC	VGH-TP	Overall	*P* value	Definitive case[a]	Clinical case[b]	Overall	*P* value
Case numbers	*N* = 78	*N* = 34	*N* = 112		79 (54/25)[c]	33 (24/9)[c]	112	0.646[d]
First aid								
Topical herbs	2	6	8		–	–	–	
Rope binding[e]	4	0	4		–	–	–	
Incision and suction	2	1	3		–	–	–	
Wound infection	52	34	86		61	25	86	
Any surgery	36	25	61		42	19	61	
Any form of bacterial culture	32	27	59		41	18	59	
Positive bacterial cultures	29	21	50		34	16	50	
Aerobic gram-positive bacteria	13	11	24		16	8	24	
Bacillus cereus	1	0	1	1	1	0	1	1
Enterococcus spp.	10	11	21	0.015	13	8	21	0.336
Coagulase -negative *Staphylococcus* species	2	2	4	0.584	3	1	4	1
Staphylococcus aureus	1	1	2	0.517	2	0	2	1
Aerobic gram-negative bacteria	27	22	49		33	16	49	
Acinetobacter baumannii	1	0	1	1	1	0	1	1
Aeromonas hydrophila	6	1	7	0.673	6	1	7	0.672
Citrobacter freundii	1	1	2	0.517	1	1	2	0.504
Escherichia coli	2	3	5	0.163	3	2	5	0.63
Klebsiella pneumoniae	0	1	1	0.304	0	1	1	0.295
Morganella morganii	17	15	32	0.016	17	15	32	0.011
Proteus spp.	4	4	8	0.242	6	2	8	1
Proteus mirabilis	1	2	3	0.218	3	0	3	0.554
Proteus penneri	1	1	2	0.517	2	0	2	1
Proteus vulgaris	3	2	5	0.638	3	2	5	0.63
Pseudomonas aeruginosa	2	4	6	0.068	4	2	6	1
Providencia spp.	4	2	6	1	3	3	6	0.358
Providencia alcalifaciens	1	1	2	0.517	1	1	2	0.504
Providencia rettgeri	3	1	4	1	2	2	4	0.58
Serratia spp.	1	2	3	0.218	3	0	3	0.554
Serratia liquefaciens	0	1	1	0.304	1	0	1	1
Serratia marcescens	1	1	2	0.517	2	0	2	1
Shewanella spp.	3	2	5	0.638	4	1	5	1
Yokenella regensburgei	1	0	1	1	1	0	1	1
Anaerobic bacteria								
Bacteroides spp.	5	2	7		5	2	7	
Bacteroides fragilis	5	2	7	1	5	2	7	1
Bacteroides uniformis	0	1	1	0.304	1	0	1	1
Fungus								
Candida parapsilosis	1	0	1	1	1	0	1	1
Polymicrobial (≥2 pathogens)	14	18	32	< 0.001	21	11	32	0.471

[a]Diagnosed by examining the snake, having the patients to identify snake through a picture, or laboratory testing of the venom

[b]Diagnosed by physical examination, serial wound inspection, a relevant history, and clinical improvement after receiving specific antivenom for *N. atra* bite

[c]The bracket number represents the cases in Taichung Veterans General Hospital/Taipei Veterans General Hospital, respectively

[d]No significant variation in the diagnostic methods between the two hospitals

[e]Included any form of rope, rubber band, or towel/clothes bindings

cally important snakes (*T. stejnegeri*, *P. mucrosquamatus*, *D. acutus*, *D. siamensis*, and *B. multicinctus*) and less toxic or nonvenomous snakes] and patients with equivocal manifestations and a negative identification of culprit snake were excluded.

3 Definition of Wound Infection

Besides purulence/abscess and organisms isolated from the fluid/tissue/blood, the appearance of certain symptoms or signs such as pain, erythema, local warmth, swelling, lymphangitis, delayed healing, malodor, crepitus in soft tissues, discolored or friable granulation tissue, or wound breakdown or dehiscence was also indicative of wound infections [16–18]. Since this was a retrospective study, we employed stricter criteria for infected wound following snakebites, which are defined as the presence of two of the following three criteria: onset of new or increasing pain, localized erythema or swelling at the bite site, or purulence at the bite site. The presence of fever and one of the above criteria also satisfied the definition of wound infection [19]. Fever is arbitrarily defined as a body temperature above 38 °C as measured with a tympanic thermometer, a device commonly used in both hospitals. If no abnormalities were mentioned in the case notes, it was assumed that no complication was present.

4 Bacteriology and Statistics

An aerobic and/or anaerobic bacterial culture was performed when infection was suspected in a snakebite wound. A deep tissue or biopsy culture was performed during surgical debridement, and blood culture was performed during febrile episodes. The culture sampling technique has been described in the literature [20]. Polymicrobial infection was defined as the growth of two or more microbes on the same infected or purulent wound [21]. Bacterial identification was performed using traditional biochemical methods with the VITEK 2 system (BioMérieux, Inc., Durham, NC, USA). Susceptibility to antimicrobial agents was determined by the disk diffusion method. Inhibition zone diameters were interpreted according to the zone diameter breakpoints recommended by the Clinical and Laboratory Standards Institute. All positive cultures were subjected to antibiotic susceptibility test analysis to maximize the test precision. The distribution of bacterial species between VGH-TC and VGH-TP and different diagnostic methods were compared using chi-square or Fisher's exact test. All data were analyzed with Statistical Package for the Social Sciences, version 22.0 (2013 release, IBM Corp. Armonk, NY, USA). A two-tailed p value <0.05 was considered statistically significant.

5 Results

Fifteen patients received first aid, including topical herbs in eight, rope binding in four, and incision and suction in three. According to the two diagnostic methods, 79 patients were diagnosed as "definitive case" (positive snake identification), including 54 at VGH-TC and 25 at VGH-TP and 33 as "clinical case" (typical manifestations), including 24 at VGH-TC and 9 at VGH-TP (Table 1). No patients received antibiotics prior to reaching the study hospitals. Clinically suspected wound infection, including cellulitis, tissue necrosis, finger or toe gangrene, or necrotizing fasciitis, developed in 86 out of 112 (77%) envenoming cases. Sixty-one (54%) patients eventually underwent various types of surgery, including local debridement, incision and drainage, fasciotomy or fasciectomy, finger or toe amputation, and skin grafting, which were all performed in the study hospitals. Bacterial cultures from any type of biological sample, including wound discharge, deep tissue or biopsy, or blood, were obtained from 59 of the 86 cases. Fifty patients (50/59, 85%) had positive bacterial cultures, and more than two organisms were isolated from 32 (32/50, 64%) patients. A total of 23 organisms were identified (Table 1). Gram-negative rod bacteria, such as members of the *Enterobacteriaceae* family, were more frequently identified than gram-positive cocci. The following pathogens were detected (in descending order): *Morganella morganii*, 32 cases; *Enterococcus* spp., 21; *Proteus* spp., 8; *Aeromonas hydrophila*, 7; and anaerobic *Bacteroides* spp., 7. *Bacteroides* spp. were the

only anaerobes implicated in these cases. Statistically, a higher incidence of *Morganella*, *Enterococcus* spp., and polymicrobial infection (≥2 pathogens) was observed at VGH-TP.

In this study, all 59 patients produced more than one set of bacterial cultures during hospitalization. Overall, 155 wound discharge, 23 deep tissue or biopsy, and 44 blood samples were obtained. Anaerobic culture was not always concomitantly performed with aerobic culture; therefore, only 47 and 2 anaerobic cultures were obtained from wound discharge and deep tissue or biopsy, respectively. The positive proportions of bacterial culture were 62.6% (97/155) in wound discharge, 78.3% (18/23) in deep tissue or biopsy, and 6.8% (3/44) in blood samples. Only members of the *Bacteroides fragilis* and *Shewanella* species were isolated from blood samples. The results of antibiotic susceptibility tests of *Enterococcus* and *Bacteroides* spp. and the most frequently occurring gram-negative pathogens are listed in Tables 2 and 3, respectively.

6 Discussion

The oral flora of snakes comprises a wide range of aerobic and anaerobic microorganisms, particularly fecal gram-negative rods, because their prey (e.g., rodents or reptiles) usually defecate while being ingested [10, 22, 23]. The bacterial compositions vary among snake species and may be influenced by venom properties [9, 19, 24] and the fecal flora of the prey in different geographic regions [23]. Laboratory bacteriological investigations of aerobic isolates from the venom and oral cavities of the North American pit viper *Crotalus atrox* demonstrated a preponderance of enteric and coliform organisms, particularly *Aerobacter*, *Proteus*, and *Pseudomonas*, with *Clostridium* as the most common anaerobic genus [25]. In the venom of *C. viridis helleri* and *C. scutulatus scutulatus*, *Proteus* spp., *P. aeruginosa*, and coagulase-negative *Staphylococcus* spp. are the most common aerobic species, and *Clostridium* is the most common anaerobic species [23]. In Thailand, *Enterobacter*, *Pseudomonas*, and *Staphylococcus* spp. are the most common aerobic species, and *Clostridium* is the most common anaerobic species in the venom or mouth of the Malayan pit viper (*Calloselasma rhodostoma*). In a recent study conducted by Shek et al. in Hong Kong, *M. morganii*, *Aeromonas hydrophila*, *Proteus* spp., *Enterococcus faecalis*, coagulase-negative *Staphylococcus*, and anaerobic *Clostridium* were the most commonly isolated pathogens in the oropharynx of *N. atra* [7].

The mouth of *N. atra* harbors larger numbers of bacterial species associated with snakebite wound infections than crotaline or colubrid snake species [7, 8]. In our study, *M. morganii* was the most predominant bacteria isolated from bite wounds, followed by *Enterococcus* spp., *Proteus* spp., *A. hydrophila*, *P. aeruginosa*, and *Providencia* spp., in descending order. Our human case study is largely consistent with the experimental findings of Shek et al. [7] with the exception of anaerobic pathogen species. *Bacteroides* spp. were the only anaerobe isolated from the *N. atra* bite wounds in our study. Notably, a previously healthy 31-year-old man developed *Bacteroides fragilis* bacteremia after a *N. atra* bite over his hand. This patient recovered after antibiotic therapy and serial wound debridement and grafting. Another previously healthy 35-year-old man developed *Shewanella* bacteremia after a *N. atra* bite over his finger. He also recovered after the administration of antibiotics, finger amputation, and grafting surgery. In both cases, polymicrobial wound infections were also present: *M. morganii*, *P. rettgeri*, *P. aeruginosa*, *Shewanella* sp., and *Enterococcus* spp. in the first case and *Enterococcus* spp., *P. mirabilis*, *P. penneri*, *Shewanella* sp., and *B. fragilis* in the second case. Although *Bacteroides* and *Shewanella* bacteremia are usually associated with an underlying immunocompromised status (e.g., malignancy), hepatobiliary disease, and high mortality rates [26, 27], the pathogenic effects of these types of bacteremia in immunocompetent patients and in the context of polymicrobial infection remain poorly understood. Snakebite may be a benign cause of *Bacteroides* or *Shewanella* bacteremia with a favorable outcome.

Table 2 Antibiotic susceptibility test of *Enterococcus* and *Bacteroides* spp. isolated from *Naja atra* bite wounds

	Ampicillin	Clindamycin	Gentamicin	Chloramphenicol	Penicillin	Sulfamethoxazole-trimethoprim	Vancomycin	Teicoplanin	Erythromycin	Metronidazole
Enterococcus spp.										
VGH-TC	17/17	–	18/18	–	18/18	–	18/18	–	–	–
(*N* = 18)[a]										
VGH-TP	13/15	3/12	9/15	12/15	14/15	10/12	15/15	15/15	7/15 (*I* = 2)	–
(*N* = 15)										
Bacteroides spp.										
VGH-TC	–	10/10	–	12/10	–	–	–	–	–	10/10
(*N* = 10)										
VGH-TP	–	6/7 (*I* = 1)	–	7/7	–	–	–	–	–	6/7 (*I* = 1)
(*N* = 7)										

VGH-TC Taichung Veterans General Hospital, *VGH-TP* Taipei Veterans General Hospital
[a]The bracket number represents the total numbers of positive culture

Table 3 Antibiotic susceptibility of the most common gram-negative pathogens isolated from *Naja atra* bite wounds[a]

Susceptibility strains	Ampicillin	Ampicillin–sulbactam	Piperacillin–tazobactam	Cefazolin	Cefotaxime	Ceftazidime	Cefmetazole	Flomoxcf	Gentamicin	Amikacin	Ciprofloxacin	Levofloxacin	Imipenem	Sulfamethoxazole-trimethoprim	Chloramphenicol
VGH-TC															
Aerobic gram-negative bacteria															
Aeromonas	0/11	0/5	11/11	0/11	11/11	5/5	5/5	4/11	11/11	11/11	11/11	5/5	6/6	10/10	5/5
hydrophila (12)[b]								(*I* = 7)[c]							
Escherichia	0/2	1/1	2/2	1/2	2/2	1/1	1/1	2/2	2/2	2/2	1/2	1/1		1/2	1/1
coli (2)															
Morganella	3/26	7/7	25/25	0/25	26/26	7/7	7/7	25/26	26/26	26/26	26/26	7/7	11/11	26/26	7/7
morganii (26)	(*I* = 4)							(*I* = 1)							
Proteus spp. (6)	1/6	3/3	6/6	0/6	6/6	3/3	3/3	6/6	6/6	6/6	6/6	3/3	1/1	6/6	3/3
Pseudomonas			8/8			8/8			7/8	7/8	6/8	6/8	8/8		
aeruginosa (8)										(*I* = 1)	(*I* = 2)	(*I* = 1)			
Providencia	4/10	0/5	11/11	0/11	11/11	5/5	5/5	11/11	11/11	11/11	11/11	5/5	6/6	11/11	2/5
spp.(11)	(*I* = 5)			(*I* = 1)											
Shewanella	1/1	3/3	3/3	0/1	1/1	3/3	1/1	1/1	3/3	3/3	3/3	3/3	2/2	3/3	1/1
spp. (3)															
VGH-TP															
Aerobic gram-negative bacteria															
A. hydrophila (1)	0/1			0/1	1/1	1/1		0/1	1/1	1/1				1/1	1/1
E. coli (3)	1/2	0/1	1/1	1/2	2/2	2/2	1/1	2/2	2/2	0/1	1/1	1/1	1/1	2/2	2/2
M. morganii (16)	3/14	10/10	13/13	3/14	14/14	14/14	10/10	14/14	14/14	9/9	13/13	5/5	13/13	14/14	14/14
	(*I* = 1)														
Proteus spp. (6)	0/6	4/4	4/4		6/6	6/6	4/4	6/6	6/6	5/5	4/4	3/3	4/4	6/6	2/6
															(*I* = 1)
P. aeruginosa (5)			5/5			5/5			5/5	4/4	5/5	2/2	5/5	0/4	1/4
Providencia	1/2	1/1	1/1	1/2	2/2	2/2	1/1	1/1	2/2	2/2	1/1		1/1	2/2	2/2
spp.(2)															
Shewanella	1/4	2/2	2/2	0/4	3/4	4/4	2/2	4/4	4/4	4/4	2/2		1/2	4/4	4/4
													(*I* = 1)		
spp.(4)															
Overall															
Aerobic gram-negative bacteria															
A. hydrophila (13)	0/12	0/5	11/11	0/12	12/12	6/6	5/5	4/12	12/12	12/12	11/11	5/5	6/6	11/11	6/6
								(*I* = 7)							
E. coli (5)	0/4	1/2	3/3	2/4	4/4	3/3	2/2	4/4	4/4	2/3	2/3	2/2	1/1	3/4	3/3
M. morganii (42)	6/40	17/17	38/38	3/39	40/40	21/21	17/17	39/40	40/40	35/35	39/39	12/12	14/14	40/40	21/21
	(*I* = 5)							(*I* = 1)							
Proteus spp. (12)	1/12	7/7	10/10	0/6	12/12	9/9	7/7	12/12	12/12	11/11	10/10	6/6	5/5	12/12	5/9

															(*I* = 1)
P. *aeruginosa* (13)			13/13			13/13			12/13	11/12	11/13	8/10	13/13	0/4	1/4
										(*I* = 1)	(*I* = 2)	(*I* = 1)			
Providencia	5/12	1/6	12/12	1/13	13/13	7/7	6/6	12/12	13/13	13/13	12/12	5/5	7/7	13/13	4/7
spp. (13)	(*I* = 5)			(*I* = 1)											
Shewanella	2/5	5/5	5/5	0/5	4/5	7/7	3/3	5/5	7/7	7/7	5/5	3/3	3/4	7/7	5/5
													(*I* = 1)		
spp. (7)															

VGH-TC Taichung Veterans General Hospital, *VGH-TP* Taipei Veterans General Hospital

[a]To maximize the precision of the antibiotic susceptibility test, all positive cultures were included in the analysis

[b]The bracket number represents the total numbers of positive culture

[c]Intermediate susceptibility

In-hospital snakebite management comprises the administration of antivenom, antibiotics for wound infections, or surgery to ameliorate infectious complications. In Brazil, Jorge et al. suggested chloramphenicol as the antibiotic of choice for the management of *Bothrops* envenomation because the most frequently isolated pathogens from these wounds include *M. morganii*, *P. rettgeri*, *Enterobacter* spp., *Escherichia coli*, *Enterococcus* spp., and *Bacteroides* spp. [28]. In Northern Thailand, Threaten et al. recommended benzylpenicillin with gentamicin as a prophylactic antibiotic regimen after Malayan pit viper (*Calloselasma rhodostoma*) envenomation because *Enterobacter* spp., *Pseudomonas* spp., and occasionally *Staphylococcus* and *Clostridia* have been cultured from the venom and mouth of this snake species [9]. However, a positive bacterial culture obtained from the mouth or venom of a snake does not necessarily correspond to a high risk of snakebite wound infection. Hence, the use of prophylactic antibiotics during snakebite management remains controversial [22]. For example, a low incidence of wound infection was documented in snakebites from certain crotaline species, despite the isolation of several pathogens from the snake venom [19, 23, 29]. Furthermore, the antibacterial effect of crotaline snake venom was previously described [7–9, 24], and prophylactic antibiotics have not been found to reduce the incidence of wound infection in prospective evaluations [30, 31].

In Taiwan, the crotaline snakes *T. stejnegeri* and *P. mucrosquamatus* cause more than 70% of all snakebite incidents [2]; however, these species rarely induce wound infections after envenomation. Chen et al. previously investigated snakebites from *T. stejnegeri* and *P. mucrosquamatus* and found that 6% and 26% cases, respectively, developed clinically suspected wound infections and 0% and 9% cases, respectively, underwent surgery, including dermatomy/fasciotomy, skin graft, and digit amputation, after envenomation [32]. In our study, 77% (86/112) of the cases developed clinically suspected wound infections, and 54% (61/112) required surgery secondary to tissue necrosis, finger or toe gangrene, and/or necrotizing fasciitis. *N. atra* venom comprises cardiotoxins, neurotoxins, hemotoxins, and phospholipase A_2, among others. Cardiotoxins and neurotoxins represent the major components and account for 55% and 10% of the dry weight of crude venom, respectively [3]. Although neurotoxins are the most lethal fraction in small mammals, they causes only mild neurotoxicity in humans; instead, the major concern in humans is cardiotoxins, which work synergistically with phospholipase A_2 to induce local tissue necrosis after snakebites, predispose the wound to bacterial infection from the indigenous oral flora of the snake, and necessitate limb amputation or cause mortality in rare circumstances [3, 33].

In our study, we identified "clinical cases" of *N. atra* bites by the typical presentations of *N. atra* envenomation. *N. atra* bites induce distinct effects, including wound necrosis (63%–100%), fever, necrotizing fasciitis, gastrointestinal effects, and systemic neurotoxicity, which are rarely or not found in crotaline (*T. stejnegeri* and *P. mucrosquamatus*) bites [3, 32, 34]. Most *N. atra* bite cases can be accurately diagnosed and treated using the diagnostic algorithm established by the Taiwan Poison Control Center, which includes physical examination, serial wound inspection, a relevant history, and clinical improvement after receiving a specific antivenom [2, 3, 15]. Only a few cases with equivocal manifestations necessitated laboratory testing of the venom to establish a definitive diagnosis [12–14]. Moreover, we did not find significant variations in the distribution of bacteriology between definitive and clinical cases, which might favor the misclassification of infected crotaline snakebite wounds into *N. atra* bites among clinical cases [7, 8].

The diagnosis of wound infection following snake envenomation remains problematic not only because the venom causes toxicological effects similar to those caused by pathogenic flora (e.g., local swelling, heat, tenderness, regional lymphadenopathy, fever, and increased white blood cell counts) [25, 35] but also because no validated physical criteria are available for the diagnosis of this particular type of wound infection [17, 19]. Nevertheless, we have tried our best to employ stricter criteria in the diagnosis of wound infection (i.e., clinical symptoms/signs

supporting the diagnosis of wound infection and organisms isolated from the wound discharge, deep tissue or biopsy, or blood). Although the incidence of wound infection might still have been overestimated in this study, we believed the overestimate was likely to be of limited magnitude given that a very high proportion of positive bacterial cultures was obtained in cases with clinically suspected infection and more than half of the patients with a diagnosis of wound infection underwent surgery because of infectious complications. Furthermore, a high incidence of wound necrosis (63%–100%), which has been recognized as a factor significantly associated with certain types of wound infection [16], was frequently observed with *N. atra* envenomation in contrast to crotaline envenomation in Taiwan [3, 32, 34]. The importance of wound infection following *N. atra* envenomation should not be overlooked. We suggested that snakebite wound infection should be considered a special wound infection entity. More objective measurements such as sonographic, laboratory, and/or validated physical criteria for snakebite wound infections should be established in the future [36–39].

The judicious use of antibiotics based on local bacteriology patterns should be considered to improve the management of *N. atra* bite wound infections. Chen et al. inspected 21 snakebite cases with wound infections and isolated at least 17 bacterial species from these wounds, including 17 caused by *N. atra*, 1 by *T. stejnegeri*, 1 by *P. mucrosquamatus*, and 2 by unknown snake species [4]. *M. morganii*, *Enterococcus* spp., and *P. aeruginosa* were the most common aerobic species and *Bacteroides* spp. the most common anaerobe species isolated from snakebites. Huang et al. analyzed 17 cases of snakebite with wound infections, including 16 caused by *N. atra* snakebite and 1 by *T. stejnegeri*, and isolated 13 bacterial species [5]. *M. morganii*, *Enterococcus* spp., and *A. hydrophila* were the most common aerobic species, and *Bacteroides* spp. were the only anaerobic species isolated in that study. Although those two studies did not specify the bacteria with respect to snake species, our findings suggest that these pathogens more likely arose from *N. atra* snakebite wounds. Accordingly, we do not recommend the routine use of antibiotics in the management of crotaline snakebites [7, 8]. In our study, no significant differences in bacterial distribution or antibiotic resistance were observed between the two hospitals, except for an increased incidence of *M. morganii*, *Enterococcus* spp., and polymicrobial infections among cases from VGH-TP, which may have been related to variations in the fecal flora of prey and oral flora of individual snakes in different geographic areas in Taiwan [40]. As *M. morganii* is naturally resistant to benzylpenicillin, aminopenicillins, oxacillin, first- and second-generation cephalosporins, and sulfamethoxazole, and given the safety profile of chloramphenicol, monotherapy with ureidopenicillin or combination therapy with aminopenicillin and a third-generation cephalosporin or fluoroquinolone may be the initial drugs of choice for the management of *N. atra* snakebite wound infection [41]. However, as increased antibiotic resistance of gram-negative bacteria to fluoroquinolone and of *Enterococcus* spp. to penicillins has been observed, we recommend the continuous surveillance of antibiotic resistance among these pathogens [42, 43].

7 Limitations

This study has several limitations. First, there is always a time delay in bacterial culture collection from snakebite wounds because of the natural course of *N. atra* envenoming [3]. Patients may have received several forms of treatment (e.g., wound cleansing, application of topical medicines, surgical debridement, or antimicrobial therapy) in a prehospital setting or during transportation or hospitalization that may have altered the bacterial composition before bacterial culture collection; therefore, the management timing cannot always be addressed in detail.

Second, in our study, anaerobic cultures were not always concomitantly performed with aerobic cultures; therefore, the incidence and numbers of cases affected by anaerobic infection may have been underestimated [22, 44].

Third, both VGH-TC and VGH-TP are referral centers; therefore, the incidence of wound infection

and the bacteriological pattern in this study may not be generalizable to all primary care facilities because of possible referral bias. Furthermore, this is a retrospective study, which suffers certain inherent limitations of the study design; hence, the results should be interpreted cautiously. Nevertheless, this is the first study to investigate a single snake species that most frequently causes snakebite wound infections in Taiwan, and the findings may have important clinical implications in the better management of *N. atra* bite.

Conclusions

A high incidence of clinically suspected wound infection was observed in cases of *N. atra* envenomation. No significant differences were observed in the distribution of bacteriology between the study hospitals, except for an increase in the incidence of *M. morganii*, *Enterococcus* spp., and polymicrobial infections at VGH-TP, which may have been related to variations in the fecal flora of prey and oral flora of individual snakes in different geographic areas in Taiwan. With the exception of anaerobic pathogens, our human case study findings support the experimental findings obtained in Hong Kong [7]. Based on the bacteriological findings, we suggest that either monotherapy with ureidopenicillin or combination therapy with aminopenicillin and a third-generation cephalosporin or fluoroquinolone is the preferred drug of choice in the initial management of *N. atra* snakebite wound infections.

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