ORIGINAL RESEARCH

Clinical Features, Bacteriology, and Antibiotic Treatment Among Patients with Presumed *Naja* Bites in Vietnam

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Introduction—Clinical and bacteriological features of cobra (*Naja*) bites are still relatively unknown in Vietnam. This study aimed to characterize the clinical and bacteriological characteristics of local wounds in patients with presumed *Naja* spp bite, as well as their antibiotic treatment.

Methods—A cross-sectional study was performed on presumed *Naja* bite patients who were admitted to Bach Mai Hospital in Hanoi, Vietnam. In vitro bacterial isolation, blood tests, and lesion measure were conducted, and antibiotic susceptibilities of localized bite wounds were assessed. The Mann-Whitney test was used to examine the difference in clinical characteristics between patients experiencing presumed *Naja atra* bites and *Naja kaouthia* bites. Data are presented as percentages or median with interquartile range, as appropriate. Statistical significance was accepted at $P < 0.05$.

Results—Among 46 patients, all had typical clinical features of *Naja* bite. The median bite-to-hospital time was 6 h (interquartile range 4.0–11.3). The dominant organisms isolated from local wounds were *Morganella morganii* (11/36) and *Enterococcus faecalis* (25/36). All cultures were susceptible to ciprofloxacin. No difference was found with regard to pain, swelling circumference, swelling spread, or necrotic area between patients bitten by presumed *Naja atra* and *Naja kaouthia* ($P > 0.05$).

Conclusions—Wound necrosis and infection were important clinical issues in presumed *Naja* spp snake bites. *Morganella morganii* and *Enterococcus faecalis* were dominant in local wound swabs of such cases. Ciprofloxacin should be an effective first-line antibiotic for patients with presumed *Naja* bite.

Keywords: snakebite, antibiotic susceptibility, *Morganella morganii*, *Enterococcus faecalis*, ciprofloxacin

Introduction

Snakebites are an important health issue in many countries with warm and humid climates. According to the World Health Organization, it is estimated that there are 5.4 million victims of snakebite each year, with half of them being associated with venomous snakes; approximately 81,000 to 138,000 deaths; and more than 100,000 people experience severe sequelae.¹ The number of people

affected by venomous snakebites in Asian countries is higher than on other continents, with around 2 million cases reported each year.¹ Venomous snakebites often lead to local wound necrosis and secondary wound infection.² Severity of venomous snakebite varies from very mild bites to severe, life-threatening envenomation, requiring initial management and emergency recovery. Treatment of venomous snakebites depends on the morphological, biological, toxicological, and clinical characteristics of each venomous snake.

In Vietnam, the 3 major *Naja* spp are *Naja atra*, *Naja kaouthia*, and *Naja siamensis*. Among these, *Naja atra* is the dominant species of cobra in Northern Vietnam.³ Annually, approximately 30,000 cases of venomous snakebite are reported, and the incidence

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of *Naja* bites is 5 per 100,000 person-years.⁴ However, these figures are likely underestimated because many cases that occur in rural areas are not reported or are treated by traditional methods. Although *Naja* bites remain common in Vietnam, wound bacteriology and antibiotic indication have not been studied. In this study, we aimed to describe the clinical and bacteriological characteristics of local wounds in patients with presumed *Naja* bite, as well as their antibiotic treatment, which may influence local clinical guidelines for snake wound infection management.

Methods

STUDY DESIGN AND SAMPLING METHOD

A cross-sectional study was conducted of patients with presumed *Naja* bites at the Bach Mai Hospital, the largest tertiary referral hospital in Hanoi, Vietnam, from September 2017 to September 2018. Patients were recruited if 1) they reported that they were bitten by a snake; 2) the snake was brought to and identified by the physicians at the poison control center of Bach Mai Hospital or patients had clinical evidence of wound infection after *Naja* spp bite occurrence; 3) specialist toxicology physicians identified patients' wounds as due to *Naja* spp bite via fang mark, swelling, and necrosis; and 4) patients agreed to participate in the study.

We applied the characteristics of snakes and wounds that were consistent with wounds observed in previous patients envenomed by *Naja* spp to identify patients with *Naja* bites. For *Naja atra*, the color of snake varies and includes brown, gray, and black. The back of the hood features 1 or 2 white marks with black dots in the middle resembling 1 or a pair of eyes, with a blackish green border. Snake venom causes soreness, local swelling, diffuse edema, and gangrene. In the first 24 h there may be blisters and watery and dark spots. For *Naja kaouthia*, the color varies from olive green to black. The back of the hood has a monocellate pattern, and the belly is usually pale yellow or green with black rings. The venom causes edema, necrosis, or other symptoms such as headache, nausea, and abdominal pain.

Patients were excluded if they 1) used prophylactic antibiotics before hospital admission or 2) refused to participate in the study. All participants brought snakes for identification (ID) verification. A toxicologist (NDN) who was familiar with *Naja* and had experience with ID of field specimens was consulted to identify the snakes. Patients provided written informed consent before enrollment. The study was approved by the institutional review board of Bach Mai hospital.

DATA MEASUREMENT

Microbiology isolation

After patients were admitted to the hospital with purulence and abscess, a sample of wound purulence was extracted by using sterile swab sticks (Huang Yu Company) and was sent to the microbiology department of Bach Mai Hospital within 1 h. The sample was isolated using the VITEK 2 system (BioMérieux, Inc., Durham, NC). Antibigram was conducted by the Kirby-Bauer method. Antibiotic susceptibility test was assessed according to the Clinical & Laboratory Standards Institute criteria.

Blood tests were conducted only when patients were admitted to the hospital. The tests included total blood count (white blood cell count [$\text{g} \cdot \text{L}^{-1}$], neutrophils [%], red blood cell count [$\text{T} \cdot \text{L}^{-1}$], hemoglobin [$\text{g} \cdot \text{L}^{-1}$], platelet [$\text{G} \cdot \text{L}^{-1}$], coagulation profile [prothrombin time (%)], activated partial thromboplastin time [s], fibrinogen, procalcitonin [$\text{ng} \cdot \text{mL}^{-1}$] and C-reactive protein ($\text{mg} \cdot \text{L}^{-1}$)).

Lesion measurement

In this study, indicators including swelling circumference (cm), swelling spread (cm), necrotic area (cm^2), and pain severity were measured. The swelling circumference was calculated as the difference between the circumference of the limb through the bite and the circumference of the healthy parallel limb. The swelling spread was defined as the distance from center of the bite to the swelling margin. Necrotic area was estimated using a grid board. Pain severity was assessed by the numeric pain rating scale. The typical symptoms of wound infection included increasing pain in the lesion area, edema, erythema, heat, purulence/abscess, purulent exudate, and delayed healing.^{5,6} The clinical severity of envenomation was assessed using the European Association of Poisons Centres and Clinical Toxicologists Poisoning Severity Score (Table 1).⁷

All patients received initial antibiotics by the on-call chief physician for prophylaxis when they were admitted to the hospital before antibiotic sensitivities were established. Appropriate initial antibiotics were defined as antibiotics used within 24 h of the first encounter when in vitro tests confirmed an existence of infection.

STATISTICAL ANALYSIS

R version 3.5.1 programming language (The R Foundation) was used to analyze the data. Median and interquartile range were used because data had nonnormal distribution. The Mann-Whitney test was used to examine the difference in clinical characteristics between patients with presumed *Naja atra* bites and *Naja kaouthia* bites. *P* values <0.05 were considered statistically significant.

Table 1. Grades of envenomation according to the European Association of Poisons Centres and Clinical Toxicologists Poisoning Severity Score

Grade	Signs and symptoms
0	Fang marks, swelling, and erythema around the fang mark <2.5 cm; minimal pain and tenderness; no systemic symptoms
1	Fang marks; history of immediate pain with the bite; swelling and erythema 5–15 cm; no systemic signs or symptoms
2	Fang marks; history of immediate severe pain; swelling and erythema 15–40 cm; mild systemic symptoms and/or abnormal laboratory findings
3	Fang marks; history of immediate severe pain; swelling and erythema >40 cm; petechiae and bullae; moderate systemic symptoms; bleeding and/or disseminated intravascular coagulopathy; abnormal laboratory values
4	Fang marks; signs of multiple envenomation sites; history of immediate severe pain; severe systemic signs, possibly including coma, shock, bleeding, disseminated intravascular coagulation, and paralysis

Results

All 46 cases had toxicity symptoms, and patients with level 2 severity were dominant (Table 2). No patient was found to be anemic, thrombocytopenic, coagulopathic, or in acute renal failure. Patients had elevated neutrophil counts, but procalcitonin and C-reactive protein levels were not increased.

Table 3 shows that no difference was found in pain ($P=0.23$), swelling circumference ($P=0.73$), swelling spread ($P=0.35$), or necrotic area ($P=0.48$) between patients bitten by presumed *Naja atra* and *Naja kaouthia*.

Positive bacterial wound cultures were found in 80% of cases. There were 48 strains of bacteria isolated from bite wound specimens taken. *Enterococcus faecalis* and *Morganella morganii* were the most common strains isolated. Cultivation results suggested that gram-positive aerobic bacterial growth was more frequent than gram-negative aerobic bacterial growth (Table 4).

Isolated *Enterococcus faecalis* was susceptible to all antibiotics. *Staphylococcus aureus* bacteria were resistant to penicillin but sensitive to methicillin. *Morganella morganii* isolates were sensitive to third- and fourth-generation cephalosporins, carbapenem, piperacillin/tazobactam, fluoroquinolones, gentamicin, and amikacin. The strains of *Enterobacter* spp, *Proteus*, *Klebsiella pneumoniae*, and *Raoultella planticola* were sensitive to third- and fourth-generation cephalosporins, carbapenem groups, piperacillin/tazobactam, fluoroquinolone, gentamicin, and amikacin. *Pseudomonas putida* was sensitive to ceftazidime, amikacin, levofloxacin, and doxycycline but resistant to meropenem (Table 5).

Most patients received initial antibiotics in accordance with antimicrobial guidelines. Two patients were recorded as having inappropriate initial antibiotic use; cultures from infected wounds were positive for *S aureus* and *Pseudomonas putida*.

Table 2. Clinical and paraclinical characteristics

Clinical variables	Median (IQR)
NRS	6 (5–7)
Swelling circumference (cm)	2 (1.0–2.3)
Necrotic area (cm ²)	10.5 (4.0–22.5)
Swelling spread (cm)	21.5 (15.0–38.8)
Envenomation grade	
1	6 (13%)
2	29 (63%)
3	10 (22%)
4	2 (2%)
Bite-to-hospital time (h)	6 (4–11)
Paraclinical variables	
RBC (T·L ⁻¹)	4.9 (4.7–5.4)
Hemoglobin (g·L ⁻¹)	150 (138–159)
Platelet (G·L ⁻¹)	239 (200–355)
PT (%)	89 (74–96)
aPTT (s)	25 (23–27)
Fibrinogen	3.6 (2.8–4.3)
WBC (G·L ⁻¹)	14.7 (8.9–17.9)
Neutrophils (%)	86 (79–90)
Procalcitonin (ng·mL ⁻¹)	0.3 (0.1–0.6)
CRP (mg·L ⁻¹)	0.5 (0.1–4.1)

aPTT, activated partial thromboplastin time; CRP, C-reactive protein; IQR, interquartile range; NRS, numeric rating scale for pain; PT, prothrombin time; RBC, red blood cell count; WBC, white blood cell count.

Discussion

In this study, we observed that most of our patients were bitten on the foot after accidentally treading on a snake or walking near a snake lair while working in the field or jungle. This result was similar to a Hong Kong study in which the authors indicated that lower limb was the most common site of bite.⁸

All patients in the present study had swelling, necrosis, and local pain typical of *Naja* spp bites. Local wound necrosis and intense pain associated with *Naja* envenomation were thought to be mediated by cytotoxins and

Table 3. Clinical characteristics between 2 types of snake

Variable	Total 46	<i>Natra atra</i> 33	<i>Natra kaouthia</i> 13	P value
NRS				
Median (IQR)	6 (5–7)	6 (5–7)	6 (6–8)	0.23
Range	4–9	4–9	4–9	
Swelling (cm)				
Median (IQR)	2.0 (1.0–2.3)	2.0 (1.0–2.5)	2.0 (1.0–2.5)	0.73
Range	1–4	1–4	1–3	
Necrotic area (cm ²)				
Median (IQR)	11 (4–23)	11 (4–17)	10 (6–45)	0.48
Range	1–300	1–200	1–300	
Swelling spread (cm)				
Median (IQR)	22 (15–39)	20 (15–31)	27 (15–52)	0.35
Range	10–78	10–78	10–65	

IQR, interquartile range; NRS, numeric rating scale for pain.

Table 4. Isolated bacteria of local wound

Cultures	n (%)
Positive cultures	36 (78)
Gram-negative	
<i>Morganella morganii</i>	11 (31)
<i>Enterobacter</i> spp	4 (11)
<i>Proteus</i> spp	2 (6)
<i>Raoultella planticola</i>	1 (3)
<i>Klebsiella pneumoniae</i>	1 (3)
<i>Pseudomonas putida</i>	1 (3)
Gram-positive	
<i>Enterococcus faecalis</i>	25 (69)
<i>Staphylococcus aureus</i>	1 (3)

proteolysis through so-called “three finger” toxins and phospholipase A2.^{9,10} In addition to pain and necrosis, swelling was very common in our study and occurred in all cases, consistent with previous studies.^{8,11,12} The median swelling circumference was approximately 2 cm greater than the corresponding site on the normal limb, and the median necrotic area and swelling spread were 11 cm and 22 cm, respectively. These features would be potentially useful to measure treatment progress. In this study, 10 of 46 patients (22%) had infection without developing an abscess. Some factors could contribute to these features, including time from the bite to hospital admission, local wound severity, and necrotic area. For the patients with severe local wound, infection, and necrosis and delayed hospital admission, we did not use antivenom serum but instead transferred them to the operating room to clean and remove necrotic tissues.

In our study, 39 of 46 patients (85%) were transferred to the hospital within 24 h after being bitten. Bite-to-hospital

time is a factor in assessing severity because toxins can enter systemic circulation within an hour.^{9–11,13} One of our patients was a 25-y-old woman who was bitten on the foot and treated with traditional medicines at home. After 72 h, she was admitted to the nearest local health facility. She was subsequently intubated and transferred to the Poison Control Center of Bach Mai Hospital and required mechanical ventilation for 2 d. The necrotic area was approximately 15×20 cm at the time of admission. Results of localized wound isolates grew *P putida*. Excision of necrotic tissues was performed 2 days after admission. She recovered well but was left with a limping gait due to lower limb local damage.

Leucocytosis and neutrophilia occurred in 32 patients at the time of admission, resulting from the initial immune response to the toxins and wound infection. *Naja* oral bacterial flora contain various aerobic and anaerobic bacteria.^{2,14} In Thailand, *Enterobacter*, *Pseudomonas*, and *Staphylococcus* spp were the most common aerobic bacteria, and *Clostridium* was the most common anaerobic bacteria in the Malayan viper (*Calloselasma rhodostoma*) oral cavity.¹⁵ In the *Naja* oral cavity, *M morganii* and *Enterococcus* are the most common bacteria.² The positive culture isolation rate was 78% (36 of 46), of which 28 of 36 (58%) were gram positive and 20 (42%) were gram negative. Eleven isolated cultures were positive for more than 1 bacterium. The dominant bacteria isolated were *E faecalis* (25 of 36) and *M morganii* (11 of 36), consistent with a study in Taiwan,¹⁵ whereas *S aureus* and *Escherichia coli* were the most common isolated bacteria in a study from India.¹⁶ This difference might be explained by differences in geography, environment, and prey. Notably, 22% had negative results,

Table 5. Antibigram with antibiotic susceptibility

Antibiotics	<i>Morganella morganii</i>	<i>Enterobacter</i> spp	<i>Proteus</i> spp	<i>Raoultella planticola</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas putida</i>	<i>Enterococcus faecalis</i>	<i>Staphylococcus aureus</i>
Ertapenem	11/11	4/4	2/2	1/1	1/1	—	—	1/1
Imepenem	11/11	4/4	2/2	1/1	1/1	—	—	1/1
Meropenem	11/11	4/4	2/2	1/1	1/1	0/1	—	1/1
Cefuroxime	11/11	4/4	2/2	1/1	1/1	—	—	1/1
Ceftazidime	11/11	4/4	2/2	1/1	1/1	1/1	—	1/1
Ceftriaxone	11/11	4/4	2/2	1/1	1/1	—	—	1/1
Cefotaxime	11/11	4/4	2/2	1/1	1/1	—	—	—
Cefepime	11/11	4/4	2/2	1/1	1/1	—	—	—
Piperacillin/ Tazobactam	11/11	4/4	2/2	1/1	1/1	—	—	1/1
Gentamicin	11/11	4/4	2/2	1/1	1/1	—	27/27	
Tobramycin	11/11	4/4	2/2	1/1	1/1	—	—	
Amikacin	11/11	4/4	2/2	1/1	1/1	1/1	—	
Ciprofloxacin	11/11	4/4	2/2	1/1	1/1	1/1	—	
Levofloxacin	11/11	4/4	2/2	1/1	1/1	1/1	—	
Trimethoprim/ Sulfamethazole	11/11	4/4	2/2	1/1	1/1	—	—	
Fosmycin	0/11	0/4	2/2	1/1	1/1	—	—	
Ampicilin	—	—	—	—	—	—	27/27	
Piperacillin	—	—	—	—	—	—	27/27	
Chloramphenicol	—	—	—	—	—	—	27/27	
Vancomycin	—	—	—	—	—	—	27/27	
Linezolid	—	—	—	—	—	—	27/27	1/1
Amoxicillin/Acid clavulanic	—	—	—	—	—	—	27/27	1/1
Ampicillin/ Sulbactam	—	—	—	—	—	—	27/27	—
Piperacillin/ Tazobactam	—	—	—	—	—	—	27/27	—

which might be because patients had minor inflammatory lesions or dry wounds leading to inoculation without bacteria.

In our study setting, early antibiotic use with a combination of clindamycin and ciprofloxacin could be indicated to prevent wound necrosis and infection. In Brazilian studies, chloramphenicol was the first-line antibiotic for *Bothrops* snakebite owing to the presence of *M. morganii*, *Providencia rettgeri*, *Enterobacter* spp, *Escherichia coli*, *Enterococcus* spp, and *Bacteroides* spp.¹⁷ Benzylpenicillin and gentamicin were used as prophylaxis for *Malayan* snakebite in which *Enterobacter* spp, *Pseudomonas* spp, *Staphylococcus*, and *Clostridium* were isolated from snakes' oral cavity.¹⁸ Piperacillin monotherapy or a combination of penicillin A with third-generation cephalosporin or fluoroquinolone were

considered first-line antibiotics for local wound infection associated with *Naja atra* bite.¹⁵ In our study, all isolated bacteria were sensitive to ciprofloxacin, and all wound infections resolved with ciprofloxacin.

There were several limitations to the study. Although this was a single-center study, cases were referred from all near provinces, making these findings generalizable to these areas as well. Second, unless the snake was identified directly, the cases were presumed to be *Naja* spp through assessment and description of the snake provided to toxicology clinicians at Bach Mai Hospital. Third, no information was collected on bacterial superinfection after therapeutic or traditional medicine intervention by a local healer or patients. In practice, snake oral flora bacteria were susceptible to most antibiotics.^{2,15}

Conclusions

Wound necrosis and infection were important clinical issues in presumed *Naja* spp snakebites. *Morganella morganii* and *Enterococcus faecalis* were dominant in local wound swabs of such cases. Ciprofloxacin should be an effective first-line antibiotic for patients with presumed *Naja* bite.

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