

CLINICAL SCIENCE

Shewanella infection of snake bites: a twelve-year retrospective study

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OBJECTIVE: Infections of snake bite wounds by *Shewanella* are rarely discussed in the medical literature. This study aims to characterize the presentation and management of *Shewanella* infections in snake bite wounds.

METHOD: We retrospectively investigated the microbiology, clinical features, and outcomes of patients with *Shewanella* infected snake bite wounds admitted to a tertiary medical center from January 1998 to December 2009.

RESULTS: Ten patients with *Shewanella*-infected snake bite wounds were identified. All of the snake bites were caused by cobras. The majority of patients had moderate to severe local envenomation and polymicrobial infections. *Shewanella* isolates are susceptible to ampicillin-sulbactam, piperacillin-tazobactam, third- and fourth-generation cephalosporins, carbapenems, aminoglycosides, and quinolones but are resistant to penicillin and ceftazidime. All of the patients examined had favorable outcomes.

CONCLUSION: It is recommended that *Shewanella* infection be considered in snake bite patients, especially when patients present with moderate to severe local envenomation.

KEYWORDS: Cobra; *Shewanella*; Snake Bites; Soft Tissue Infections; Wound Infection.

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INTRODUCTION

Snake bite envenomation is a common medical emergency in the tropics (1). An estimated 421,000 envenomings result from snake bites each year worldwide and that these envenomings cause 20,000 deaths annually (2). Moreover, many patients suffer permanent disabilities from wound complications (3-5). Bacterial wound infection is a major cause of wound complications. As many as 30.8% of snake bite victims suffer wound infections (6). While serious systemic illnesses occur infrequently as a result of these infections, they can lead to sepsis and death (7,8).

Various organisms have been reported to cause infections after snake bites. Polymicrobial infections are seen frequently, with *Enterobacteriaceae* being among the most common isolates (9). A recent study of snake bite wound

infection reported the isolation of species in the *Shewanella* genus from 3 patients; however, the species of snake involved and patient details were not provided (10). *Shewanella* is a non-fermentative Gram-negative bacteria that occurs worldwide (11). In a surveillance study of snake oral bacteria in Hong Kong, *Shewanella* was present in the oropharynx of the Chinese cobra (*Naja atra*) (12).

Few studies have reported on *Shewanella* infections; those that have consist mostly of isolated case reports and case series (13). Soft tissue infection is the most commonly described presentation in human infections (14). There is little published data on *Shewanella* infections in association with snake bites.

Shewanella is probably an underestimated and under-reported cause of wound infections following snake bite. We therefore sought to determine the clinical features of *Shewanella* infection through a retrospective review of the microbiology records of patients with snake bites seen over a 12-year period.

MATERIALS AND METHODS

The medical records of patients with *Shewanella*-infected snake bite wounds who presented to the Taichung Veterans

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No potential conflict of interest was reported.

General Hospital, a tertiary medical center in Taiwan, between January 1998 and December 2009 were reviewed. Patients were identified using the institution's electronic database. Clinical, laboratory, and microbiology data were retrieved from the patients' medical records. Two independent researchers reviewed and confirmed the medical records.

The identification of the snake species involved in each case was based on: 1) inspection of the snake if it had been brought to the hospital; or 2) the patient's identification of the snake using a venomous snake chart and the corresponding clinical manifestations.

The effects of the snake bite on the surrounding tissue were classified into three categories of clinical severity as defined in previous studies: 1) minimal- swelling, erythema, or ecchymosis confined to the site of the bite, 2) moderate- progression of swelling, erythema, or ecchymosis beyond the site of the bite, or 3) severe- rapid swelling, erythema, or ecchymosis involving the entire limb. This classification is in accord with the severity classification system proposed by Gold et al. (15).

The method for sampling wound tissue was adopted from previous studies (16,17). A deep tissue culture obtained by biopsy during the initial debridement was performed. Specimens obtained from the open necrotic area, the draining of abscesses and hematomas were not included because of possible contamination.

The identification of *Shewanella* was based on Gram staining, colony morphology, and biochemical characteristics.

Antibiotic susceptibility testing was performed using a disk-diffusion technique involving the use of Oxoid disks according to the Clinical and Laboratory Standards Institute guidelines.

The study protocol was approved by the Institutional Review Board of the Taichung Veterans General Hospital. (C10257).

RESULTS

Ten patients were identified as having a snake bite wound infected with *Shewanella*. Each patient had been bitten by a cobra, one of six medically important venomous snakes in Taiwan; other venomous snakes in Taiwan include *Trimeresurus mucrosquamatus*, *Trimeresurus stejnegeri*, *Bungarus multicinctus*, *Deinagkistrodon acutus*, and *Daboia russelii siamensis* (18).

All of the patients presented with local symptoms typical of cobra bites. Pain, erythema and swelling were noted in all cases, as well as characteristic dark discoloration around the fang mark. Nine of the patients had necrotic wounds at the time of presentation. The local reaction to envenomation was rated moderate to severe in nine of the patients (Table 1). Four patients, who had been bitten in the upper portion of their limb, developed compartment syndrome. None of these patients developed symptoms of systemic poisoning, such as changes in consciousness, paralysis, or paresis. The median age of the patients was 42.5 years (range, 26-88 years). Two of the patients had hypertension,

Table 1 - Clinical characteristics of 10 patients with a *Shewanella* infected wound from a cobra bite.

Case no.	Age in years, Sex	Underlying condition	Month of presentation	Site of the bite	Delay between bite and treatment (hours)	Symptoms at presentation	Local severity of envenomation	Compartment syndrome	Other organisms isolated	Antibiotic therapy
1	39, M		April	Left hand	18	P, E, S, B, N	Severe	Yes	<i>Morganella morganii</i> , <i>Providencia rettgeri</i> , <i>Enterococcus</i>	ciprofloxacin
2	88, F	Hypertension	July	Left foot	32	P, E, S	Moderate	No	<i>Morganella morganii</i>	ceftriaxone
3	48, F		August	Left foot	16.5	P, E, S, B, N	Severe	No	<i>Morganella morganii</i> , <i>Bacteroides fragilis</i> , <i>Enterococcus</i>	ampicillin/ sulbactam
4	63, M	Hypertension	August	Right hand	23	P, E, S, N	Severe	Yes	<i>Aeromonas hydrophila</i> , <i>Enterococcus</i>	ampicillin/ sulbactam
5	38, M		July	Right hand	2.5	P, E, S, N	Severe	Yes		ampicillin/ sulbactam
6	46, M		May	Left hand	4	P, E, S, N	Moderate	Yes	<i>Morganella morganii</i>	ceftriaxone
7	38, F		June	Right foot	2	P, E, S, N	Moderate	No	<i>Bacteroides fragilis</i> , <i>Providencia stuartii</i>	ceftazidime, metronidazole
8	29, F		October	Left foot	1	P, E, S, N	Moderate	No	<i>Providencia rettgeri</i>	ampicillin/ sulbactam
9	72, F		October	Right foot	5	P, E, S, B, N	Moderate	No	<i>Proteus vulgaris</i>	cefoxitin
10	26, F		July	Right foot	4	P, E, S, B, N	Mild	No		amoxicillin/ clavulanate acid

P, pain; S, swelling; E, erythema; B, bullous; N: necrosis.

but the other eight patients had no underlying disease. All of the cases occurred between April and October. Six of the patients were bitten while working on farms. The bites were localized to the lower limbs in six of the patients; the bites in the remaining patients were localized in the upper limbs. The mean time that elapsed after the bite and prior to arrival at the hospital was 10.8 hours (range 1-32 hours).

Two patients had monomicrobial infections, while eight had concomitant polymicrobial infections. The concomitant pathogens isolated included: *Morganella morganii* (four patients), *Providencia* species (three patients), *Enterococcus* species (three patients), *Bacteroides fragilis* (two patients), and *Aeromonas hydrophila* (one patient) and *Proteus vulgaris* (one patient).

All of the patients received anti-venom treatment and underwent wound debridement. With regard to antimicrobial treatment, most of the patients received broad-spectrum beta-lactam antibiotics, including ampicillin/sulbactam (four patients), ceftriaxone (two patients), amoxicillin-clavulanate (one patient), ceftazidime (one patient), and cefoxitin (one patient). One patient received ciprofloxacin. The majority of patients experienced prolonged hospitalization with an average stay in the hospital of 23.5 days. All of the infections were treated successfully.

All of the *Shewanella* isolates tested were susceptible to ampicillin-sulbactam, piperacillin-tazobactam, third- and fourth-generation cephalosporins, carbapenems, aminoglycosides, and quinolones (Table 2). Ninety percent of the isolates tested were susceptible to trimethoprim-sulfamethoxazole and chloramphenicol, while 83% were susceptible to ampicillin. All of the *Shewanella* isolates tested were resistant to penicillin and cefazolin.

DISCUSSION

This study reports 10 cases of *Shewanella* infection in wounds resulting from cobra bites. Among them, nine had moderate to severe local envenomation, and four patients in this group experienced compartment syndrome. *Shewanella* isolates in the study were characteristically susceptible to most third- and fourth-generation cephalosporins and piperacillin but resistant to penicillin and cefazolin.

Cobra bite is a major cause of morbidity and mortality in Asia (19). Furthermore, wound infection is a common and

severe complication of cobra bite (10). In a retrospective study performed over a 10-year period, Chen et al. reported that cobra bites cause more wound infections than any other type of venomous snake and result in more severe complications (10). Death due to necrotizing fasciitis after a cobra bite has been reported (8). Our results suggest that the patients who had *Shewanella*-infected wounds were likely to have been bitten by a cobra. The frequent occurrence of complications from infection at the site of a cobra bite is the result of the synergistic effects of envenomation and contamination with pathogenic bacteria. Cobra venom contains cytotoxins, which are responsible for the swelling and tissue necrosis that spread rapidly after envenomation (20). When humans are envenomated, the cytotoxins released induce enzymatic destruction of the endothelium and connective tissue, which results in necrosis at the site of the bite and produces an ideal environment for bacterial proliferation. Upon envenomation, a potent inoculum of bacteria is introduced by the snake's fangs. Previous studies have shown that wound infections develop more frequently in moderate to severe snake bite wounds (21), like those experienced by the majority (9/10) of our patients.

Cobra venoms are neurotoxic, hemotoxic and cardiotoxic (22). However, local necrosis is the most prominent clinical feature of cobra bites in many parts of Asia; neurotoxic manifestations are uncommon (20,23). Our findings are in agreement with previous observations, as none of our patients had neurological symptoms or signs. Another study of cobra bites in Sri Lanka found that local symptoms were the only manifestation in most cases (4). The varied presentation of patients after cobra envenomation may reflect geographical variation in the composition of cobra venom (24). The mainstay of treatment for cobra envenomation is the administration of anti-venom (25). However, published studies have failed to demonstrate that anti-venom administration has a significant effect in controlling local tissue necrosis and its sequelae at the site of the bite (4,20).

Four of the cobra-bitten patients in our study developed compartment syndrome. These patients were all bitten on the upper limbs. *Shewanella* was unlikely the sole cause of the compartment syndrome. *Morganella morganii*, *Aeromonas hydrophila*, *Providencia rettgeri*, and *Enterococcus* were also isolated from these patients and may have contributed to the compartment syndrome. The venom-induced tissue destruction may have prompted the development of compartment syndrome, leaving the wound susceptible to *Shewanella* infection (23).

The source of the bacteria found in these wound infections may be bacteria inhabiting the snake's oral cavity (26-28). In a survey of snake oral bacterial flora in Hong Kong, cobras harbored many types of bacteria, including enteric and coliform organisms (12,29). *Shewanella* has been found within the oral cavities of Chinese cobra specimens (12). Thus, these animals are a likely source of the bacteria that caused the wound infections in our patients. The affinity of *Shewanella* for necrotic tissue has been reported (30-32). Necrosis is an important feature of cobra bites and provides an ideal environment for the growth of *Shewanella*.

Our data also support previous observations that polymicrobial infections are common both in infected snake bite wounds (9) and *Shewanella* infections (33). Because *Shewanella* infections are uncommon and because these

Table 2 - Antimicrobial susceptibility data for the *Shewanella* isolates.

Antimicrobial agent	No. of susceptibility isolates (%)
Ampicillin-sulbactam	10/10 (100)
Piperacillin-tazobactam	10/10 (100)
Cefotaxime	6/6 (100)
Ceftazidime	10/10 (100)
Cefepime	9/9 (100)
Imipenem	7/7 (100)
Aztreonam	6/6 (100)
Amikacin	10/10 (100)
Gentamicin	10/10 (100)
Minocycline	6/6 (100)
Ciprofloxacin	8/8 (100)
Trimethoprim-sulfamethoxazole	9/10 (90)
Chloramphenicol	9/10 (90)
Ampicillin	5/6 (83)
Cefazolin	0/6 (0)
Penicillin	0/8 (0)

bacteria are often isolated with other organisms, the clinical significance of these infections has been obscured. More recently, increased numbers of monomicrobial *Shewanella* infections have been reported, and this type of infection may contribute to a more fulminant clinical course. Moreover, animal studies have confirmed the pathogenicity of *Shewanella* (34). *Shewanella algae* and *Shewanella putrefaciens* are the only two species of the genus known to cause human infections (14). *Shewanella* is often a component of a polymicrobial infection. The most common clinical syndrome caused by *Shewanella* is an infection of the skin and soft tissue (35). Hepatobiliary diseases and malignancy are the most common underlying diseases in cases of *Shewanella* infection, which is in contrast to the patients described in this study, most of whom (8/10) were in good health before the injury. The overall mortality rate for *Shewanella* infections can be as high as 20 to 30%; this high mortality may be due to severe underlying disease in many cases (13). All of the patients reported in our study that presented with acute *Shewanella* wound infections survived to discharge. This result was due in part to early diagnosis, which made prompt treatment possible. The lack of any underlying disease is another possible cause of the favorable outcomes in our patients.

As with other cases of bacterial wound infection, definitive treatment should be based on antimicrobial susceptibilities and involve adequate surgical debridement. Antimicrobial susceptibility data for our isolates and our clinical results are consistent with those described by other investigators. Specifically, *Shewanella* is characteristically susceptible to ampicillin-sulbactam, piperacillin-tazobactam, third- and fourth-generation cephalosporins, carbapenems, aminoglycosides and quinolones but resistant to penicillin and cefazolin (13,36). These data suggest that initial therapy for *Shewanella* infection could include a broad-spectrum beta-lactam antibiotic or a fluoroquinolone. Broad-spectrum beta-lactam antibiotics and fluoroquinolones are also effective against other common pathogens associated with snake bites. Ninety percent of isolates were susceptible to chloramphenicol, which is often used in the treatment of infected wounds from snake bites (37). However, penicillin and cefazolin, which are often recommended for the empiric treatment of infected wounds from venomous snakes other than cobras (16,38), have poor activity against *Shewanella*. In our opinion, physicians should consider the species of venomous snake involved when selecting an appropriate antibiotic.

Although *Shewanella* is susceptible to many antibiotics, the existence of carbapenem-resistant strains has been reported (33). Furthermore, Lascols et al. have reported that a chromosomally encoded gene, *qnr3*, may confer resistance to fluoroquinolones in *Shewanella* (39). The emergence of resistance to piperacillin-tazobactam (40) and imipenem (41) during treatment has been reported. The failure of treatment should alert the clinician to the possibility of emerging resistance.

One limitation of our study is that it reported detailed information from only one tertiary care center. In that sense, our data set does not represent a general patient population and is subject to biases regarding referrals for advanced care. Patients with mild cases are probably underrepresented.

Second, we did not identify the isolated *Shewanella* samples at the species level. Because *S. algae* is not included

in the databases used by many of the automated identification systems, many clinical microbiology laboratories identify both species as *S. putrefaciens* (34). Thus, a number of the infections reported as *S. putrefaciens* may actually correspond to *S. algae* infections. Furthermore, only molecular characterization can definitively distinguish *S. putrefaciens* and *S. algae* from other species of the *Shewanella* genus (35).

Third, snake species identification was not confirmed by herpetologists due to the nature of a retrospective study. The identification of a snake species by doctors, victims, and witnesses can be unreliable (42). Immunodiagnostic methods for snake identification have been proposed (1). Some experts advocate another approach; the use of diagnostic algorithms based on a previously established snake bite database (42).

In conclusion, this study provides further evidence that *Shewanella* is a pathogen associated with snake bite wound infections, especially in patients with moderate to severe local envenomation. The use of broad-spectrum beta-lactam antibiotics and debridement are associated with good outcomes. Several issues require further study. The entire spectrum of wound infections after snake bite requires close investigation. This topic may be best investigated by prospective integral observation studies. A comprehensive survey of the bacterial oral flora found in venomous snakes in this region is urgently needed.

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AUTHOR CONTRIBUTIONS

The work presented here was carried out as a collaboration among all the authors. Liu PY, Tung KC and Shi ZY defined the research theme. Liu PY, Tung KC and Lin CF designed the methods and experiments, carried out the laboratory experiments, analyzed the data, interpreted the results and wrote the paper. Chan KW and Huang JA worked together on data collection and interpretation. Liu JW and Shi ZY co-designed the experiments and discussed the analysis, interpretation, and presentation. All authors have read and approved the manuscript.

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