ORIGINAL ARTICLE

Oral bacterial flora of the Chinese cobra (*Naja atra*) and bamboo pit viper (*Trimeresurus albolabris*) in Hong Kong SAR, China

KC Shek 石錦全
KL Tsui 徐國樑
KK Lam 林家強
Paul Crow 高保然
Kenneth HL Ng 吳浩良
Gary Ades 艾加里
KT Yip 葉錦棠
Alessandro Grioni
KS Tan 陳結山
David C Lung 龍振邦
Tommy SK Lam 林成傑
HT Fung 馮顯達
TL Que 郭德麟
CW Kam 甘澤華

Objective To determine the oral bacterial flora associated with two common local venomous snakes in Hong Kong, namely the Chinese cobra (*Naja atra*) and the bamboo pit viper (*Trimeresurus albolabris*).

Design Cross-sectional study.

Setting A non-government organisation and a regional hospital in Hong Kong.

Subjects Thirty-two Chinese cobras and seven bamboo pit vipers.

Main outcome measures Species identification of bacteria in the oral cavity of both snakes and their antibiotic susceptibilities.

Results The oral cavity of Chinese cobra harbour a wide range of pathogenic bacteria, including: Gram-negative bacterial species like *Morganella morganii*, *Aeromonas hydrophila* and *Proteus*, and Gram-positive bacteria like *Enterococcus faecalis*, coagulase-negative *Staphylococcus* as well as anaerobic species (clostridia). The oral cavity of the Chinese cobra is more likely than that of the bamboo pit viper to harbour pathogenic bacteria associated with snakebite infection (P<0.001). The median number of pathogenic bacteria per snake was significantly higher in the Chinese cobra (P<0.001). All pathogenic Gram-negative bacteria isolated were susceptible to levofloxacin. Amoxicillin/clavulanate provided good coverage against pathogenic Gram-positive bacteria (*Enterococcus faecalis*) and anaerobes.

Conclusion 'Prophylactic' antibiotic treatment for Chinese cobra bites may be beneficial, owing to the multiple pathogenic bacteria in its oral cavity and the higher risk of ensuing necrosis. The regimen of levofloxacin plus amoxicillin/clavulanate appears promising for this purpose, but further study is required to confirm its clinical utility in patients.

Key words
Abscess; Antibiotic prophylaxis; Snake bites; Wound infection

Hong Kong Med J 2009;15:183-90

Tuen Mun Hospital, Hospital Authority, Hong Kong:
Accident and Emergency Department
KC Shek, FRCS (Edin), FHKAM (Emergency Medicine)
KL Tsui, FRCS (Edin), FHKAM (Emergency Medicine)
KK Lam, MRCS(Ed), FHKAM (Emergency Medicine)
TSK Lam, MB, BS, MRCS(Ed)
HT Fung, MRCP, FHKAM (Emergency Medicine)
CW Kam, MRCP, FHKAM (Emergency Medicine)
Department of Pathology
KHL Ng, MRCP, FHKAM (Pathology)
KT Yip, MMedSc
DC Lung, MB, BS
TL Que, FRCPath, FHKAM (Pathology)
Fauna Conservation Department, Kadoorie Farm and Botanic Garden, Hong Kong
P Crow, BSc
G Ades, BSc, PhD
A Grioni, DVM, MRCVS
KS Tan, BSc

Correspondence to: Dr KC Shek
E-mail: shekkc@yahoo.com.hk

Introduction

Envenoming due to snakebite is not an uncommon emergency in Hong Kong[1,2]; 80 patients with snakebites (both venomous and non-venomous) were admitted into local hospitals in 2007.[3] Although Hong Kong is generally regarded as an urbanised region, a significant proportion of the countryside still supports healthy snake populations, especially in the New Territories. Wound infection is a known complication after snakebites. Venomous snakebites often result in local wound necrosis, and necrotic tissue is an excellent medium for bacterial growth. Local wound infections or abscesses can occur in such patients, especially when there is an associated haematoma or necrosis at the bite site.[4] Many bites are on the limbs of young and otherwise healthy individuals. The resulting functional impairment and loss of productivity are substantial.

Knowledge of the bacterial flora acquired from snakebites constitutes important information pertinent to management. There have been numerous studies overseas to determine the oral flora in snakes[4-6] and the bacteriology associated with snake venom,[7] but for Hong Kong specific data are lacking. In this study, we set out to characterise and compare the oral bacterial flora of the two of the most common venomous snakes in this locality, namely the Chinese cobra (*Naja atra)* and the bamboo pit viper (*Trimeresurus albolabris*). Based on such findings, possible implications for antibiotic treatment could be suggested.

中國香港特別行政區內的眼鏡蛇與白唇竹葉青蛇的口腔細菌群落

目的 確定香港境內的眼鏡蛇（*Naja atra*）與白唇竹葉青蛇（*Trimeresurus albolabris*）的口腔細菌群落。

設計 橫斷面研究。

安排 香港一所非政府機構和一所地區醫院。

對象 32條眼鏡蛇及7條白唇竹葉青蛇。

主要結果測量 兩種蛇口腔內的細菌種類及其耐藥性。

結果 眼鏡蛇口腔內有多種不同的病原菌，包括革蘭氏陰性細菌（如摩氏摩根氏菌、嗜水氣單胞菌、變形桿菌屬）、革蘭氏陽性細菌（如糞腸球菌、凝固酶陰性葡萄球菌），以及厭氧菌（梭狀芽胞桿菌屬）。與白唇竹葉青蛇比較，眼鏡蛇口腔內的病原菌與被蛇咬而受感染更有關係（P<0.001）；而每條眼鏡蛇的病原菌平均數量亦明顯較多（P<0.001）。所有分離出的革蘭氏陰性細菌均對左氧氟沙星呈敏感，而阿莫西林和克拉維酸可產生保護作用來阻隔革蘭氏陽性病原菌（糞腸球菌）及厭氧菌。

結論 眼鏡蛇口腔內的細菌種類多不勝數，加上可能引致的壞死症，所以對被眼鏡蛇咬傷的傷者施以預防性抗生素治療可能有用。左氧氟沙星加上阿莫西林／克拉維酸治療似乎可以治療蛇咬，但必須進行更多研究以確定其臨床療效。

Methods

Background and setting

Kadoorie Farm and Botanic Garden (KFBG) is a non-government organisation funded by the Kadoorie Foundation. It has played an active role in promoting and conserving biodiversity, sustainable agriculture, and creative nature education in Hong Kong and Southern China. In Hong Kong, all stray snakes (with the exception of Burmese pythons) handled by the Hong Kong Police Force are sent to the KFBG for species identification and health assessment before being released back into the wild. The snakes are transferred separately in a cloth bag and enclosed within a wooden box. Snakes are not given any food, drugs, or antibiotics. The present study targeted two venomous snakes only, namely: the Chinese cobra and the bamboo pit viper. Snakes other than these and unhealthy specimens were excluded. This study was approved by the New Territories West Cluster Clinical and Research Ethics Committee of the Hospital Authority and Animal Ethics Committee of the KFBG.

Study period

During the study period (21 June to 4 September 2007), 2 processing days were set every week. On each day, a medical team was sent to provide on-site medical support to the snake experts of the KFBG.

Swabbing procedure

Snakes were taken from their storage bags by the snake handling team and manually restrained throughout the exercise. If required the veterinary surgeon opened the mouth of the snake utilising a sterile mouth gag to facilitate acquisition of the oral swabs. Two oropharyngeal swab samples were collected from each snake using proprietary commercial sterile cotton-tipped swab sticks. Swabs were taken by rotating the cotton tip on the floor of the oral cavity between the larynx and mandibular teeth. One sample was kept in a Stuart's transport medium tube and the other in Robertson's cooked meat medium (RCM). The mouth gag was sterilised between each swabbing procedure to avoid cross-contamination. Any unsatisfactory or contaminated sample was discarded. Within 2 hours of the swabbing procedure, the swab samples were delivered to the Tuen Mun Hospital's Microbiology Laboratory (Department of Clinical Pathology) for processing.

Aerobic and anaerobic culture

In the laboratory, samples in Stuart's transport media were plated immediately on blood agar with 5% horse blood (BA), a chocolate agar plate (CHO), a MacConkey agar plate (MAC), and a fastidious anaerobic agar plate with 5% horse blood (FAA) [Lab M, Bury, England]. The BA, CHO, and MAC plates were incubated at 37°C with 5% CO_2 for up to 48 hours, and the FAA plates at 37°C anaerobically for 48 hours. The RCM samples were sub-cultured to the FAA after incubation for 24 hours at 37°C in ambient air, and the FAA was incubated anaerobically at 37°C for 48 hours.

Bacterial identification

The aerobic and facultative anaerobic isolates were primarily identified on the basis of their colonial morphology, Gram and acid-fastness characteristics, growth requirements, motility tests, pigment production, and standard conventional biochemical and phenotypic tests. The API (bioMérieux, Marcy l'Etoile, France) and/or Phoenix automated microbiology system (BD Diagnostic Systems, Sparks [MD], US) were used. The manufacturers' instructions were followed for further speciation of the isolates whenever the results of conventional methods were inconclusive. All anaerobes were tested with the RapID ANA II system (Innovative Diagnostic Systems, Atlanta [GA], US) for bacterial identification. Isolates with ambiguous or atypical biochemical profiles were subjected to rDNA sequencing using the MicroSeq

BOX. Pathogenic bacteria known to cause snakebite wound infections or abscesses[8-13]

Aerobic Gram-positive bacteria
- Beta-haemolytic streptococci*
- Coagulase-negative *Staphylococcus*
- *Enterococci*
- Group D *Streptococcus**
- *Staphylococcus aureus**
- *Streptococcus viridans*

Aerobic Gram-negative bacteria
- *Aeromonas hydrophila*
- *Citrobacter diversus**
- *Citrobacter freundii*
- *Enterobacter aerogenes**
- *Enterobacter agglomerans**
- *Enterobacter* species
- *Escherichia coli*
- *Morganella morganii*
- *Proteus* species
- *Providencia rettgeri*
- *Pseudomonas pseudoalcaligenes**
- *Salmonella arizonae**
- *Serratia liquefaciens**
- *Serratia* species
- *Yersinia enterocolitica**

Anaerobic bacteria
- *Bacteroides* species

* Not found in our study

500 16S rDNA Bacterial Identification Kit (Applied Biosystems, Foster City [CA], US) according to the manufacturer's instructions. The sequences were compared with the GenBank database using the Basic Local Alignment Search Tool (BLAST) search. Antimicrobial susceptibility tests were performed on the isolates, according to the Clinical and Laboratory Standard Institute's guidelines. Antibiotic susceptibility tests were not performed on anaerobic bacteria.

Definition of pathogenic bacteria

Among the bacteria isolated from the snake's oral cavity, not all should be considered pathogenic (liable to result in wound infections or abscesses) in humans. Some were environmental contaminants or plant pathogens only. Others had never been reported to cause any snakebite wound infection. A literature search was carried out to identify the types of bacteria reported to have caused soft-tissue infection or abscesses in snakebite wounds; and in this study these were then defined as potentially pathogenic (Box).[8-13]

Statistical analysis

Descriptive statistics were employed to show the frequency of different bacteria. Comparison of categorical data and continuous data was carried out using Chi squared/Fisher's exact tests and the Mann-Whitney test, respectively.

Results

A total of 39 healthy stray snakes native to Hong Kong were processed during the study period. They included 32 Chinese cobras and seven bamboo pit vipers.

The oropharynx of the Chinese cobras sampled contained a wide range of bacteria; 10 were aerobic Gram-positive species, 20 aerobic Gram-negative species, and 14 anaerobic species (Table 1). Among Gram-negative bacteria, *Morganella morganii* was the commonest pathogen. Other important Gram-negative pathogens included *Aeromonas hydrophila* and *Proteus* species. *Enterococcus faecalis* and coagulase-negative staphylococci were the commonest Gram-positive isolates. Various anaerobic *Clostridium* species were also found.

The oropharynx of the bamboo pit vipers sampled contained a modest range of bacteria; three were aerobic Gram-positive species, nine were aerobic Gram-negative species, and three were anaerobic species (Table 2). Potentially pathogenic bacteria (coagulase-negative staphylococci, *Citrobacter freundii*, *Proteus vulgaris*, and *Bacteroides eggerthii*) were found in three bamboo pits vipers. Streptococcal species and *Staphylococcus aureus* were not isolated.

As a result, only three of these seven snakes yielded one or more potential pathogens, while all Chinese cobras yielded one or more potentially pathogenic bacteria. Notably, the Chinese cobras appeared more likely to have pathogenic bacteria in their oral cavity than the bamboo pit vipers; this difference being statistically significant ($P<0.001$, 2-tailed Fisher's exact test). The median number of pathogenic bacteria per snake was three and zero for Chinese cobras and bamboo pit vipers, respectively; the proportion being significantly higher in the Chinese cobras ($P<0.001$, 2-tailed Mann-Whitney test).

Susceptibilities of the isolated pathogenic Gram-negative bacteria to common antibiotics are shown in Table 3. All Gram-negative bacteria were susceptible to levofloxacin and netilmicin. Not all pathogenic Gram-negative bacteria were susceptible to cefuroxime or amoxicillin/clavulanate. Among Gram-positive bacteria in our study, all *E faecalis* isolates were susceptible to ampicillin and vancomycin. Moreover, four of the seven isolates of coagulase-negative staphylococci were methicillin-resistant, but they were all susceptible to gentamicin, trimethoprim/sulfamethoxazole, amikacin, vancomycin, rifampin, and levofloxacin.

Discussion

This study was performed during the summer and early autumn when the weather in Hong Kong is

TABLE 1. Species of aerobic Gram-positive and Gram-negative bacteria, as well as anaerobic bacteria isolated from the oropharynx of the 32 Chinese cobras in our study

Organism	No.
Aerobic Gram-positive bacteria	63
Bacillus species	3
Coagulase-negative staphylococci	6
Diphtheroid bacillus	3
Enterococcus faecalis	27
Enterococcus gallinarum	3
Micrococcus species	2
Rhodococcus species	1
Streptococcus viridans	1
Tsukamurella species	15
Vagococcus species	2
Aerobic Gram-negative bacteria	85
Achromobacter denitrificans	10
Acinetobacter haemolyticus	1
Aeromonas hydrophila complex	4
Bordetella trematum	2
Chryseobacterium indologenes	1
Chryseobacterium meningosepticum	2
Citrobacter freundii	4
Enterobacter cloacae	1
Escherichia coli	2
Klebsiella planticola	1
Klebsiella pneumoniae	3
Morganella morganii	23
Proteus mirabilis	1
Proteus penneri	6
Proteus vulgaris	4
Providencia rettgeri	3
Pseudomonas aeruginosa	8
Serratia marcescens	5
Shewanella putrefaciens group	2
Stenotrophomonas maltophilia	2
Anaerobic bacteria	54
Bacteroides fragilis	2
Clostridium baratii/sardiniense	11
Clostridium bifermentans	13
Clostridium butyricum	2
Clostridium glycolicum	3
Clostridium innocuum	1
Clostridium lituseburense	1
Clostridium perfringens	7
Clostridium ramosum	1
Clostridium sordellii	4
Fusobacterium nucleatum	1
Fusobacterium varium	2
Prevotella loescheii	1
Prevotella oralis group	5

TABLE 2. Species of bacteria isolated from the oropharynx of the seven bamboo pit vipers in our study

Organism	Total
Aerobic Gram-positive bacteria	5
Bacillus species	1
Coagulase-negative staphylococci	1
Diphtheroid bacillus	3
Aerobic Gram-negative bacteria	14
Acinetobacter lwoffii	1
Chryseobacterium indologenes	1
Chryseobacterium meningosepticum	1
Citrobacter freundii	1
Proteus vulgaris	1
Pseudomonas aeruginosa	3
Rhizobium radiobacter	2
Sphingobacterium paucimobilis	1
Stenotrophomonas maltophilia	3
Anaerobic bacteria	2
Bacteroides eggerthii	1
Clostridium baratii/sardiniense	1

warm and snakes are usually more active, which also coincides with increased human outdoor activities (trail walking and camping). During these parts of the year, the chances of human-snake encounters are significantly increased, which probably explain why most snakebites encountered in Hong Kong occur during summer and early autumn.[2,14]

The bamboo pit viper and Chinese cobra are responsible for the majority of venomous snakebites in Hong Kong,[1,2,15] and are the two most common venomous snakes sent to the KFGB for identification. Other than systemic manifestations, bites due to these two species can result in local wound swelling and necrosis at the involved site, which predispose to secondary bacterial infection including abscess formation. At least one case of fatal necrotising fasciitis after a Chinese cobra bite has been reported in Hong Kong.[15]

The oral bacterial flora of snakes varies between different species as well as at different geographical regions.[6] Regarding the pattern of oral isolates from the snakes in this study, findings were similar to those from other overseas.[4-6] Goldstein et al[7] suggested that the oral flora of snakes appears to be faecal in nature, as the live prey may defecate in the snake's mouth coincident with ingestion.[7] Notably, our findings agree with previous findings from Hong Kong indicating a low wound infection rate from bamboo pit viper bites, there being no wound infections or abscesses in a series of local cases reported in 1993.[16] By contrast, in one series more than half of all cobra bites resulted in skin necrosis or an infected wound.[13] Based on our knowledge of their natural history, the

TABLE 3. Susceptibility of Gram-negative bacteria isolated in our study to common antibiotics

Antibiotic	Susceptible strains, No. (%)							
	Morganella morganii (n=29)*	*Escherichia coli* (n=2)	*Proteus* species (n=12)	*Aeromonas hydrophila* (n=4)	*Enterobacter* species (n=1)	*Serratia* species (n=5)	*Citrobacter freundii* (n=5)	*Providencia rettgeri* (n=3)
Gentamicin	28 (97)	2 (100)	12 (100)	4 (100)	1 (100)	5 (100)	5 (100)	3 (100)
Netilmicin	29 (100)	2 (100)	12 (100)	4 (100)	1 (100)	5 (100)	5 (100)	3 (100)
Trimethoprim/ sulfamethoxazole	27 (93)	1 (50)	12 (100)	4 (100)	1 (100)	5 (100)	5 (100)	3 (100)
Levofloxacin	29 (100)	2 (100)	12 (100)	4 (100)	1 (100)	5 (100)	5 (100)	3 (100)
Cefuroxime sodium	13 (45)	2 (100)	8 (67)	4 (100)	1 (100)	0	5 (100)	3 (100)
Cefuroxime axetil	10 (34)	0	8 (67)	3 (75)	0	0	2 (40)	3 (100)
Amoxicillin/clavulanic acid	6 (21)	1 (50)	11 (92)	0	1 (100)	0	1 (20)	1 (33)
Amikacin	29 (100)	2 (100)	12 (100)	4 (100)	1 (100)	5 (100)	5 (100)	3 (100)
Ceftazidime	29 (100)	2 (100)	12 (100)	4 (100)	1 (100)	5 (100)	5 (100)	3 (100)
Piperacillin/tazobactam	29 (100)	2 (100)	12 (100)	4 (100)	1 (100)	5 (100)	5 (100)	3 (100)
Cefoperazone/sulbactam	29 (100)	2 (100)	12 (100)	3 (75)	1 (100)	5 (100)	5 (100)	3 (100)
Imipenem	29 (100)	2 (100)	12 (100)	4 (100)	1 (100)	5 (100)	5 (100)	3 (100)

* Six Chinese cobra snakes contained two strains of *Morganella morganii*

differences in habitat, the hunting strategy, and the type of prey selected by these two species provide an explanation for the observed differences in oral flora, though further research is required to confirm this proposition. On the other hand, it is known that some snake venoms have anti-bacterial properties.[17,18] One postulation could be that there were differences in anti-bacterial activities between the venoms of the two snakes, but this requires further study.

Aerobic Gram-positive bacteria

In this study, the most common pathogenic aerobic Gram-positive bacteria encountered were *E faecalis* and coagulase-negative staphylococci. Enterococci rarely cause cellulitis or other deep tissue infections. In surgical wound infections, they are frequently isolated from mixed cultures with Gram-negative bacilli and anaerobes, decubitus ulcers, and diabetic infections.[19] All enterococcal isolates in our study were susceptible to ampicillin and vancomycin. Coagulase-negative staphylococci often presents in clinical specimens as a culture contaminant. In snakebite infections, it was reported as a rare pathogen in comparison to Gram-negative bacteria.[8,9,12] No *S aureus* was isolated from the snakes in our study. Though uncommon, this pathogen has been reported to cause snakebite abscesses.[8,9]

Aerobic Gram-negative bacteria

Among all the aerobic Gram-negative bacteria identified, *M morganii* was the most frequent and worthy of special clinical attention. It was reported to be the main offending organism in many bacteriological studies involving cases of snakebite wound abscesses.[10,12] More than two thirds of the Chinese cobras (23/32) in this study had this pathogen in their oral cavities. Envenoming by Chinese cobra may cause extensive tissue damage and devitalisation that can predispose the wound to bacterial infection. All isolates of *M morganii* in our study were sensitive to levofloxacin, gentamicin, and trimethoprim/sulfamethoxazole, but more than 50% were resistant to cefuroxime and more than 80% to amoxicillin/clavulanate. Interestingly, in this study no *M morganii* were isolated from any bamboo pit vipers. This was in contrast to the findings of Suankratay et al[20] who reported that they were the commonest Gram-negative bacteria in bamboo pit vipers in Thailand. This contrary result could be partly explained by the relatively small number of bamboo pit vipers (only seven) in our study, but may represent a genuine difference related to preferences for prey in different regions.

Pseudomonas aeruginosa is a known human pathogen causing skin and soft-tissue infections (folliculitis, ecthyma gangrenosa in neutropenic patients, and in burn wounds).[21] However, it has seldom been reported in snakebite wound infections or abscesses, for which its significance remains uncertain. A less common but important potential pathogen is *A hydrophila*, which was only found in the oral cavity of the Chinese cobra (4/32) and not in any bamboo pit viper in this study. *Aeromonas hydrophila* is known to cause diarrhoeal illness as well as soft-tissue infection after minor trauma coupled with exposure to fresh water.[22] It has been reported to cause soft-tissue infection as a complication of bites from snakes with local necrotising, myotoxic, and oedema-inducing venom.[10] All *Aeromonas* isolates in our study were susceptible to levofloxacin and

gentamicin but resistant to amoxicillin/clavulanate. In some studies, other enteric Gram-negative bacteria (*Escherichia coli*, *Enterobacter* species, *Citrobacter* species, and *Proteus* species) have been reported to be important offending bacteria.[8,9] However, they were infrequently identified in our study, and all were susceptible to levofloxacin.

Anaerobic bacteria

Although no *Clostridium tetani* were isolated in our study, in several others tetanus has been reported as a life-threatening complication from snakebites.[6,23] Suankratay et al[20] also did not identify *C tetani* in the oral cavity of bamboo pit vipers. The origin of the tetanus bacilli could be the snake's venom or oral cavity, the victim's own skin, non-sterile dressings or instruments applied to the bite wound and soiling of the wound after injury. Habib[23] described four snakebite patients complicated by tetanus, all of whom had their bite sites incised by instruments and medicinal herbs applied. Tetanus prophylaxis is still recommended to prevent this potentially lethal disease. Besides, isolates of *Clostridium perfringens* were found in the oral cavities of Chinese cobras in this study. Nevertheless, their role in causing bite wound infection or gas gangrene remains in doubt; there being no reported case of clostridial myonecrosis caused by snakebite wound infection in the literature. Moreover, anaerobic bacteria causing snakebite wound infection are uncommon.[12]

Insight into the use of antibiotic

Routine use of 'prophylactic' antibiotics in the treatment of snakebite is controversial. In the past, it was suggested that broad spectrum of antibiotics were indicated for most patients following venomous snakebite.[24,25] In recent years, numerous researchers disagree on the routine use of 'prophylactic' antibiotics under such circumstances,[12,26-29] and currently most guidelines do not recommend routine use of antibiotics for these patients.[30-33] However, 'prophylactic' antibiotics should still be considered in venomous snakebites resulting in significant tissue necrosis.[12] In Hong Kong, most patients do receive 'prophylactic' antibiotics following venomous snakebites[14,15,34]; the commonest regimen being ampicillin plus cloxacillin.[16] The findings of this study pose a challenge to local practice on several grounds. First, we showed that local bamboo pit viper's oral cavities had significantly fewer pathogenic bacterial species than those of Chinese cobras. Second, the number of pathogenic bacteria isolated from local bamboo pit vipers was small. Third, a significant proportion of pathogenic bacterial isolates (especially those of *M morganii*, *A hydrophila*, and *E coli*) were resistant to amoxicillin/clavulanate but susceptible to levofloxacin. Instead of routine 'prophylaxis' with ampicillin plus cloxacillin for all venomous snakebite victims, we suggest using levofloxacin plus amoxicillin/clavulanate for cobra snakebite wounds. For these patients, early use of antivenom is advocated in order to prevent tissue necrosis and enable adequate tissue antibiotic concentration to be achieved. The rationale of such a regimen is to cover most enteric Gram-negative bacteria, including *M morganii* by levofloxacin. Gram-positive bacteria (enterococci and staphylococci) and anaerobes should be adequately covered by amoxicillin/clavulanate. Levofloxacin plus metronidazole can be used in patients with β-lactam allergy, as this combination will cover most aerobic and anaerobic organisms. Routine use of vancomycin as the 'prophylactic' antibiotic for cobra bite is not recommended. Knowing that bamboo pit vipers harbour significantly fewer pathogens in their oral cavities and that their venom seldom causes severe local tissue destruction predisposing to wound infection, we suggest not using 'prophylactic' antibiotics for bite wounds from this snake.

For established snakebite wound infections, empirical use of levofloxacin plus amoxicillin/clavulanate will provide coverage for most of pathogenic bacteria. Further changes to the regimen will depend on the patient's clinical course and response to treatment as well as laboratory culture and sensitivity results. For pregnant victims, child victims, and those with special host factors like immunosuppression, a microbiologist should be consulted.

Limitations

This is the first study in Hong Kong to describe the oral bacterial flora in the two most commonly encountered native species of venomous snakes. Our findings shed light on the rationale for 'prophylactic' antibiotics to reduce snakebite wound infections, but our study had several limitations. First, with respect to bamboo pit vipers the sample size was small, for which reason less common but important pathogens could have been missed. Second, our study was conducted during summer and early autumn, but the pattern of bacterial flora in the oral cavity of snakes may change depending on the season and availability of prey. Third, our suggestions for the use of 'prophylactic' antibiotics and empirical treatment of established infections are based on the assumption that only the most common pathogens need to be covered. Many factors may affect the actual chance of incurring clinically significant wound infections in victims, including: host factors, the presence of tissue necrosis, and secondary environmental contamination.

Conclusion

The oropharynx of the wild Chinese cobras and

bamboo pit vipers in Hong Kong contains a wide range of bacteria; Gram-negative organisms being the commonest. In Hong Kong, the Chinese cobra is more likely to have pathogenic bacteria in its oral cavity than the bamboo pit viper. 'Prophylactic' antibiotics appear to be more indicated for Chinese cobra bite wounds than for those due to the bamboo pit vipers. The regimen of levofloxacin plus amoxicillin/clavulanate appears promising for both prevention as well as empirical treatment of Chinese cobra–bite wound infections, but further studies are required to confirm the clinical utility of such treatment.

Acknowledgements

The authors thank Dr Wai-neng Lau and Ms Noira Chan of KFBG for their assistance. We are also indebted to all the medical and nursing staff of the Accident and Emergency Department of Tuen Mun Hospital (Dr Oi-fung Wong, Dr Ching-him Choy, Dr Ying-leung Mak, Dr Wai-ming Ching, Dr King-yue Li, Mr Yuk-lung Wai, Mr Chun-kwong Cheung, Mr King-pong Wong, Ms Mei-yi Chan, Ms Ah-yung Chan, and Ms Po-ki Chan) who provided on-site emergency care during the swabbing procedure. Lastly, we send special thanks to the staff of the Microbiology Laboratory of Tuen Mun Hospital for their advice and support on the project.

Declaration

This is to declare that this study and part of the content of this manuscript has been submitted by the first author to the University of Hong Kong for partial fulfilment of the requirement for the Postgraduate Diploma in Infectious Diseases.

References

1. Fung HT, Lam KK, Kam CW. Efficacy and safety of snake antivenom therapy: experience of a regional hospital. Hong Kong J Emerg Med 2006;13:70-8.
2. Ng WS, Cheung WL. Snake bites in Hong Kong: a prospective study on epidemiology and pre-hospital management. Hong Kong J Emerg Med 1997;4:68-73.
3. Clinical Data Analysis and Reporting System, Hospital Authority, Hong Kong. Accessed 1 Jun 2008.
4. Blaylock RS. Normal oral bacterial flora from some southern African snakes. Onderstepoort J Vet Res 2001;68:175-82.
5. Goldstein EJ, Agyare EO, Vagvolgyi AE, Halpern M. Aerobic bacterial oral flora of garter snakes: development of normal flora and pathogenic potential for snakes and humans. J Clin Microbiol 1981;13:954-6.
6. Theakston RD, Phillips RE, Looareesuwan S, Echeverria P, Makin T, Warrell DA. Bacteriological studies of the venom and mouth cavities of wild Malayan pit vipers (*Calloselasma rhodostoma*) in southern Thailand. Trans R Soc Trop Med Hyg 1990;84:875-9.
7. Goldstein EJ, Citron DM, Gonzalez H, Russell FE, Finegold SM. Bacteriology of rattlesnake venom and implications for therapy. J Infect Dis 1979;140:818-21.
8. Nishioka Sde A, Silveira PV. Bacteriology of abscesses complicating bites of lance-headed vipers. Ann Trop Med Parasitol 1992;86:89-91.
9. Kerrigan KR. Bacteriology of snakebite abscess. Trop Doct 1992;22:158-60.
10. Jorge MT, Ribeiro LA, da Silva ML, Kusano EJ, de Mendonça JS. Microbiological studies of abscess complicating Bothrops snakebite in humans: a prospective study. Toxicon 1994;32:743-8.
11. Jorge MT, Nishioka Sde A, de Oliveirá RB, Ribeiro LA, Silveira PV. *Aeromonas hydrophila* soft-tissue infection as a complication of snake bite: report of three cases. Ann Trop Med Parasitol 1998;92:213-7.
12. Blaylock RS. Antibiotic use and infection in snakebite victims. S Afr Med J 1999;89:874-6.
13. Liao WB, Lee CW, Tsai YS, Liu BM, Chung KJ. Influential factors affecting prognosis of snakebite patients management: Kaohsiung Chang Gung Memorial Hospital experience. Chang Gung Med J 2000;23:577-83.
14. Chan TY, Critchley JA. An epidemiological study of the snake bites in the New Territories East, Hong Kong. Ann Trop Med Parasitol 1994;88:219-21.
15. Cockram CS, Chan JC, Chow KY. Bites by the white-lipped pit viper (*Trimeresurus albolabris*) and other species in Hong Kong. A survey of 4 years' experience at the Prince of Wales Hospital. J Trop Med Hyg 1990;93:79-86.
16. Chan TY, Chan JC, Tomlinson B, Critchley JA. Clinical features and hospital management of bites by the white-lipped green pit viper (*Trimeresurus albolabris*). Southeast Asian J Trop Med Public Health 1993;24:772-5.
17. Talan DA, Citron DM, Overturf GD, Singer B, Froman P, Goldstein EJ. Antibacterial activity of crotalid venoms against oral snake flora and other clinical bacteria. J Infect Dis 1991;164:195-8.
18. Blaylock RS. Antibacterial properties of KwaZulu natal snake venoms. Toxicon 2000;38:1529-34.
19. Moellering RC Jr. *Enterococcus* species, *Streptococcus bovis* and *Leuconostoc* species. In: Mandell GL, Bennett JE, Dolin R, editors. Principles and practice of infectious diseases. 6th ed. Philadelphia: Elsevier; 2005: 2411-21.
20. Suankratay C, Wilde H, Nunthapisud P, Khantipong M. Tetanus after white-lipped green pit viper (*Trimeresurus albolabris*) bite. Wilderness Environ Med 2002;13:256-61.
21. Pier GB, Ramphal R. *Pseudomonas aeruginosa*. In: Mandell GL, Bennett JE, Dolin R, editors. Principles and practice of infectious diseases. 6th ed. Philadelphia: Elsevier; 2005: 2587-615.
22. Steinberg JP, Del Rio Carlos. Other gram-negative and gram-variable bacilli. In: Mandell GL, Bennett JE, Dolin R, editors. Principles and practice of infectious diseases. 6th ed. Philadelphia: Elsevier; 2005: 2751-68.
23. Habib AG. Tetanus complicating snakebite in northern Nigeria: clinical presentation and public health implications. Acta Trop 2003;85:87-91.
24. Gold BS, Barish RA. Venomous snakebites: current concepts in diagnosis, treatment, and management. Emerg Med Clin North Am 1992;10:249-67.
25. Forks TP. Evaluation and treatment of poisonous snakebites.

Am Fam Physician 1994;50:123-30,135.
26. Kerrigan KR, Mertz BL, Nelson SJ, Dye JD. Antibiotic prophylaxis for pit viper envenomation: prospective, controlled trial. World J Surg 1997;21:369-73.
27. Tagwireyi DD, Ball DE, Nhachi CF. Routine prophylactic antibiotic use in the management of snakebite. BMC Clin Pharmacol 2001;1:4.
28. LoVecchio F, Klemens J, Welch S, Rodriguez R. Antibiotics after rattlesnake envenomation. J Emerg Med 2002;23:327-8.
29. Jorge MT, Malaque C, Ribeiro LA, et al. Failure of chloramphenicol prophylaxis to reduce the frequency of abscess formation as a complication of envenoming by Bothrops snakes in Brazil: a double-blind randomized controlled trial. Trans R Soc Trop Med Hyg 2004;98:529-34.
30. Juckett G, Hancox JG. Venomous snakebites in the United States: management review and update. Am Fam Physician 2002;65:1367-74. Erratum in: Am Fam Physician 2002;66:30.
31. Gold BS, Barish RA, Dart RC. North American snake envenomation: diagnosis, treatment, and management. Emerg Med Clin North Am 2004;22:423-43.
32. Junghanss T, Bodio M. Medically important venomous animals: biology, prevention, first aid, and clinical management. Clin Infect Dis 2006;43:1309-17.
33. Gold BS, Dart RC, Barish RA. Bites of venomous snakes. N Engl J Med 2002;347:347-56.
34. Hon KL, Kwok LW, Leung TF. Snakebites in children in the densely populated city of Hong Kong: a 10-year survey. Acta Paediatr 2004;93:270-2.