

## Isolation of gram-negative bacteria from metacarpal injury of *Panthera tigris sumatrae* trapped in Subulussalam, Indonesia

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**Abstract.** The present study aimed to isolate gram-negative bacteria infected metacarpal of *Panthera tigris sumatrae* trapped in Subulussalam, Aceh Province. Swab sample was obtained from metacarpal injury of *P. tigris sumatrae*. Swab sample was cultured to nutrient broth media using sterile cotton swabs or Pasteur pipettes, and incubated at 37°C temperature for 24 hours. Culture was spread on MacConkey media and incubated again at 37°C temperature for 24 hours. Determination of bacteria colony growth on the surface of MacConkey media based on shape, colour, surface, size, and viscosity (consistency). The bacteria colony stained with Gram staining, and tested biochemically. The result showed that gram-negative bacteria such as *Citrobacter sp.*, *Proteus sp.*, *Providencia sp.*, *Pseudomonas sp.*, and *Salmonella sp.* isolated from metacarpal injury of *P. tigris sumatrae*.

**Key words:** *Panthera tigris sumatrae*, negative Gram, bacteria.

### Introduction

Wounds can be classified as accidental, pathological or post-operative. Whatever the nature of the wound, infection is the attachment of microorganisms to host cells and they proliferate, colonize and become better placed to cause damage to the host tissues. Development of wound infection depends on the interplay of many factors. The breaking of the host protective layer the skin, and thus disturbing the protective functions of the layer, will induce many cell types into the wound to initiate host response. Individuals who have a suppressed immune system are particularly susceptible to opportunistic infections (Carter, 2008).

Open wounds are prime targets for infection, as are areas of the body where high bacteria populations are already present, like the digestive system and respiratory system. Infection can move below the skin, forming an abscess. Infection of the wound is the successful invasion, and proliferation by one or more species of microorganisms anywhere within the body's sterile tissues, sometimes resulting in pus formation (Brown, 2011). Wound can be infected by a variety of bacteria ranging from gram-positive to gram-negative bacteria (Bowler et al., 2001). Wounds, particularly deep puncture wounds, that are contaminated with dirt are the most likely to become infected such as tetanus (a serious disease that infects both humans and animals) (Brown, 2011). The common aerobic pathogens in cat bites include facultatively anaerobic, Gram-negative, nonmotile, non-spore-forming, pleomorphic coccobacillus is the most common organism isolated in cat bites (Thomas and Brook, 2011). Infections in wildlife such as tiger can even affect the muscles or blood, resulting in a deadly condition known as sepsis.

When a large number of bacteria get into a wound, it can get infected. Infection begins when an organism successfully colonizes by entering the body, growing and multiplying. Entrance to the host generally occurs through the port de entry including open wounds. While a few organisms can grow at the initial site of entry, many migrate and cause systemic infection in different organs. Some pathogens grow within the host cells (intracellular) whereas others grow freely in bodily fluids (Brown, 2011). There are different types of bacteria. More than one type may infect animal wound at the same time. Normal bacteria that lives on animal skin often enter a wound first. A break in the skin gives them a chance to enter it and cause infection. Bacteria may also come from the environment, such as soil, air, or water. If an object such as a nail caused the wound, bacteria may come from that. If wound are bit by an animal, it's saliva (spit) can also cause infection.

Microorganism on which most Gram-negative, reflecting its importance as a common opportunistic pathogen. It is often associated with infections of immunocompromised patients, such as suffering from severe wounds. Carter (2008) report that the basis of the pathogenicity of gram-negative bacteria is the ability to produce and secrete multiple extracellular virulence factors such as proteases, haemolysins, exotoxin, exoenzyme etc.. These exofactors are collectively capable of causing extensive tissue damage in animals.

Most bacteria are characterized by having not only a cell membrane but also a cell wall which lies outside of the cell membrane. This cell wall is composed mostly of peptidoglycan and helps to maintain osmotic pressure and the cell's characteristic shape. Some taxonomic groups of bacteria also have an outer membrane that is attached to the peptidoglycan by small lipoprotein molecules. This difference in outermost cell structure is the basis for classification of bacteria by a differential staining technique known as the Gram stain. Gram-positive cells (those without an outer membrane) stain purple in the procedure, gram-negative cells (which have the outer membrane) stain red or pink.

Characterization of the bacteria recovered at the time of initial tissue injury could influence the selection of empiric antimicrobial agents used to prevent infection. Here, we investigate Gram-negative bacteria infection wound in muscle metacarpal is injury of *Panthera tigris sumatrae* caused by trap. We hypothesized that not only normal bacteria of less virulence but also occasionally and pathogenic bacteria would be found to contaminate wounds immediately after wounding. In this study, we characterize the bacteriological features of metacarpal wound near the time of injury before amputation. These data may potentially shape the empiric choice of antimicrobial agents to adequately control contamination and to prevent future infection.

## **Materials and Methods**

### **Case report**

A one year old female tiger, *P. tigris sumatrae* was lose all of it's fingers on hand right due to the accidental tripping of traps trapped in Desa Sikarabang Transmigrasi SP2, Kecamatan Longkip, Subulussalam, Aceh Province. The tiger was saved in Badan Konservasi Sumberdaya Alam (BKSDA) Banda Aceh. The patient's medical history was unremarkable, except for a some days history of performance of feline posture look like thin. The large feline presented to the BKSDA some days after sustaining the feline trapped. Because of concerns about metacarpal injury and fingers lose, feline was transferred to emergency care clinics of Veterinary Medicine Faculty of Syiah Kuala University, where patient amputated, especially on metacarpal of hand right all fingers.

### **Source of swabs**

Swabs were collected from metacarpal wound swabs of *P. tigris sumatrae* sent for examination to the microbiology laboratory of Veterinary Faculty of Syiah Kuala University. Swab samples were cultured to nutrient broth media and incubated at 37°C temperature for 24 hours.

### **Bacteriological procedures**

Aerobic cultures should include inoculation of sheep blood agar for general growth. MacConkey agars are slightly selective and differential plating media mainly used for the detection and isolation of gram-negative organisms from clinical sources. MacConkey Agar is used for isolating and differentiating lactose fermenting from lactose-nonfermenting gram-negative enteric bacilli. Cultures are incubated in humid air at 36°C for 48 hours. Cultures are examined each day for growth and any colonies are Gram stained and subcultured (i.e., transferred) to appropriate media. All isolates were identified according to the methods advocated by Edwards and Ewing (1962). The specific methods involved were colonial characteristics on media including size, inability to swarm, ability or inability to ferment lactose. Specific tests such as carbohydrate utilization tests, indole formation, Methyl Red, Voges Proskauer and citrate tests were done.

### **Grams stain**

The usual first step in any bacterial identification is the determination of whether or not it is a gram-positive or gram-negative bacterium. Here, we obtain a clean glass slide to prepare a smear of each bacteria to be stained by taking a loopful of the bacteria (with a sterile loop) and spreading it over a small area in the center of the slide. The slide allowed the smear to air dry and then heat fix by passing the slide quickly through a flame. The slide

placed on paper towels and add a drop or two of crystal violet to the smear to let set 1 minute. The slide washed gently the stain off with tap water carefully in order to being not to wash off bacteria. Then, Gram's iodine apply to let set 1 minute. To remove any excess stain or stain that has not adhered to the cell, the slide washed gently the iodine off with tap water and then add the decolorizing agent (95% EtOH) drop by drop until it runs clear. The decolorizing reagent washed off with tap water, and counterstain with safranin by adding 1-2 drops and let it set for 45 seconds. Finally, the slide rinsed with tap water, looked at under the microscope, and determined if bacterium is Gram-positive or Gram-negative. Gram-negative cells will pick up the counterstain and appear red or pink as described by Health Protection Agency (2007).

## Results and Discussion

Swab samples were positive for spesies of the Gram-negative bacteria isolated from metacarpal wound of *P. tigris sumatrae* as shown in Table 1.

Table 1. Gram-negative bacteria isolated from metacarpal wound of *P. tigris sumatrae*

Test	Isolate				
	A	B	C	D	E
Shape	b	b	b	b	b
Motility	+	+	+	+	+
Aerob	+	+	+	+	+
H <sub>2</sub> S	+	-	-	+	+
Indol	-	+	+	-	-
Methyl Red	+	+	+	+	+
Voges Proskauer	-	-	-	-	-
Citrate	+	+	+	+	+
Glucose	+	+	+	+	+
Lactose	+	-	-	-	-
Mannitol	+	+	+	-	+
Maltose	-	-	-	-	-
Sucrose	-	-	-	-	-

Note: A = *Citrobacter sp.*, b = bacil, B = *Proteus sp.*, + = positive reaction, C = *Providencia sp.*, - = negative reaction, D = *Pseudomonas sp.*, E = *Salmonella sp.*

In this study, only five Gram-negative bacteria were detected, namely: *Citrobacter sp.*, *Proteus sp.*, *Providencia sp.*, *Pseudomonas sp.*, and *Salmonella sp.* isolated from metacarpal injury of *P. tigris sumatrae* (please see Table 1). This result indicate that bacteria contaminate wounds and thus cause infections. Infections occur when the population of bacteria in a wound becomes too much for the body's immune system to handle. Goldstein et al. (1984) reported that wound infections often contain multiple organisms including both aerobic and anaerobic gram-positive cocci and gram-negative bacilli and yeast. The most common pathogens isolated from wounds are: *Streptococcus* Group A, *Staphylococcus aureus*, *Escherichia coli*, *Proteus*, *Klebsiella*, *Pseudomonas*, *Enterobacter*, Enterococci, *Bacterioides*, *Clostridium*, *Candida*, *Peptostreptococcus*, *Fusobacterium*, and *Aeromonas*.

The patient in this case, a tiger was cut it's metacarpal due to for some days the accidental tripping of traps. There are possibility many situations where infection can occur. The bacteria involved tend to originate from the oral cavity of the patient because the large feline may be bite the hand to find out of traps, as well as the environment where the injury occurred. The chance of a wound becoming infected is dependent upon the nature, size, and depth of the wound; its proximity to and involvement of nonsterile areas such as the skin directly connecting to soil appear the opportunity for organisms from the environment to enter the wound. Organisms which are non-pathogenic can become pathogenic given opportunistic conditions, and even the most virulent organism requires certain circumstances to cause a compromising infection. So, in infected wounds replicating organisms exist and tissue is injured.

The present study has demonstrated that the *Citrobacter* species infected wound from large feline. Evidence that *Citrobacter* species not only found in wound of wild life animals but also involved in wound infection of survivors who attacks by sharks. Interaminense et al. (2010) showed that 81 potential bacterial pathogens were identified in the oral cavity of sharks involved in attacks in Recife. The majority were Enterobacteria such as *Proteus* spp., *Providencia alcalifaciens*, including *Citrobacter* spp. isolated from the wounds of a shark victim. As with other bite-associated infections, those due to cats are polymicrobial, with a mix of aerobes and anaerobes (Thomas and Brook, 2011).

Here, we successfully isolated *Proteus* sp. in wound muscle of metacarpal injury of *P. tigris sumatrae*. The *Proteus* group belongs to the family of Enterobacteriaceae. As members of the Enterobacteriaceae, these *Proteus* species are part of the gram negative bacilli. Although *Proteus* sp. are frequently recovered from infected wounds, Mordi and Momoh (2009) reported that *Proteus* species were the commonest coliforms isolated from wound infections in various parts of body from patients suffering of accidental, trauma, pathological and post-operative wounds. *Proteus mirabilis* was the most commonly isolated, followed by *Proteus vulgaris*, *Proteus rettgeri*, and *Proteus morgagni* from patients in the University of Benin Teaching Hospital. Indeed, the genus *Proteus* is found in soil, water, and faecally contaminated materials.

The result in the present study has observed that the *P. tigris sumatrae* is infected with a wide variety of bacterial species in trapped wound including species of *Providencia*. These organisms reported by Montgomery et al. (2002) that wounds inflicted by the dragon during encounters with prey species are also associated with *Providencia* species infection in the prey animal.

*Pseudomonas* species was found in this investigation. This is in line to the observations of Kehinde et al. (2004) who claimed that *Pseudomonas* species was the most predominant in burn wounds after *Klebsiella* species. *P. aeruginosa*, commonly associated with lung infections. Thomas and Brook (2011) reported that multiple microorganisms, especially aerobic Gram negatives, have been isolated from wounds after crocodiles (alligator) attacks. A study of the oral flora of alligators showed *Pseudomonas* is one of species to be the predominant aerobic isolates.

Previous study in line with our result regarding the wound pathogen infections often contain multiple organisms including aerobic gram-negative bacilli such as *Bacillus* sp., *Citrobacter* sp., *Proteus* sp., *Providencia* sp., and *Pseudomonas* sp. (Goldstein et al., 1984; Isotalo et al., 2000; Montgomery et al., 2002; Mordi and Momoh, 2009; Interaminense et al., 2010, and Thomas and Brook, 2011). This is however contrary to our observations about finding the *Salmonella* sp. involved in wound on metacarpal of hand right from large feline. Here, important to note that the patient failure to adequately clean the wound, along with delayed medical care, often leads to infection which are usually due to contamination from the environment.

The finding of the present study supported by previous cases. Isotalo et al. (2000) reported that zoonotic infections developing after large feline bites. A 35 year old, previously healthy man was bitten once on his left palm by an adult Siberian tiger (*P. tigris altaica*) and who developed infectious tenosynovitis. Some bacteria were successfully isolated, containing two other Gram negative bacilli from cultures of the wound. Montgomery et al., (2002) investigated saliva as a potential source of pathogenic bacteria in wild and captive Komodo dragons (*Varanus komodoensis*), the largest living lizard. Amount 28 Gram-negative including *Citrobacter* sp., *Proteus* sp., *Providencia* sp., and *Pseudomonas* sp., and 29 Gram-positive species of bacteria were isolated from the saliva of the 39 Komodo dragons. Although trapped wound of large felines are rare, it is important to characterise it's wound flora and to identify bacterial pathogens from trapped wounds and also important tools in the management of wound infections especially those caused by bacteria to provide guidelines for useful in formulating rational antibiotic policy. On the other hand, the conclusion of our study is indicative that special attention should be given to the prevention and management of Gram-negative bacterial infections in trapped wound of tiger.

## Conclusions

Wound on metacarpal injury of *P. tigris sumatrae* due to the accidental tripping contaminated with gram-negative bacteria, namely: *Citrobacter sp.*, *Proteus sp.*, *Providencia sp.*, *Pseudomonas sp.*, and *Salmonella sp.*

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