Strategical Treatment of a Tiger Affected by Multidrug-Resistant Bacteria in Chattogram Zoo

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Abstract

Antimicrobial resistance has become an alarming threat in the new world for animal and human populations, and it grows due to overuse or uncontrolled use of the antibiotic in the livestock sector. The largest cat species, the tiger, is native to Asia and the national animal of Bangladesh. In Chattogram Zoo a tiger was injured by the fighting, and a wound formed on the shoulder region. Firstly, it was treated with the antibiotic flucloxacillin (10 mg/kg body weight) intramuscularly for 13 days, but when it did not respond to treatment, bacteriological isolation and antimicrobials susceptibility (AMS) tests flucloxacillin was found resistant. The animal was then threated with Cefazolin (20 mg/kg body weight) intramuscularly for 13 days after lab test but the wound was not recovered. Then again treated with the other sensitive antibiotic, ciprofloxacin (15 mg/kg body weight), intramuscularly for 12 days without improvement. Finally, the tiger was treated with the antibiotic, linezolid, at a dose of 600 mg at 12-hour intervals for 15 days, and then the tiger was fully recovered.

Keywords: Wound, Antibiotic, AMR, CS Test, Sensitive, Linezolid

Chapter 1: Introduction

Tiger is the largest cat species, and it is native to Asia (Maharjan et al., 2024). In taxonomical classification, it belongs to the order Carnivora within the family Felidae (Wild Cat Family., 2024). There are nine subspecies of tiger in the world; six of these subspecies are endangered, and the rest three are extinct (Wilting et al., 2015). The endangered six subspecies are the Bengal, Indo-Chinese, South China, Siberian, Malayan, and Sumatran tigers. The extinct three species are the Caspian tiger (*Panthera tigris virgata*), the Javan tiger (*Panthera tigris sondaica),* the Bali tiger *(Pantera tigris balica)* (Liu et al., 2018). The Bengal Tiger is one of the living subspecies that is native to Asia, most commonly in Bangladesh, India, Nepal, and Bhutan (Goodrich et al., 2022). It is the national animal of Bangladesh. A vast population of the Bengal tiger lives in the Sundarbans, in Bangladesh. According to the last census conducted in 2024, the number of tigers living in the Sundarbans is 125 (Bangladesh Forest Department, 2024). The Bengal tiger is listed as endangered on the IUCN Red List of Threatened Species (IUCN Red List of Threatened Species, 2022). Zoos provide conservation support for endangered species. So zoos play a vital role in the conservation of the tiger population (Christie et al ., 2010).

Antimicrobial resistance (AMR) has become one of the top global threats for humans, animals, and plants (World Health Organization, 2023). It occurs when bacteria can escape the antimicrobial effect of antibiotics through multiple types of mechanisms. Some bacteria can nutrientize the components of antimicrobials and inactive this antibiotic; some can export the antibiotic out of cells, and others can change receptors for antibiotics by modifying their structure (Habboush et al., 2023). According to the WHO, antimicrobial resistance is the resistance of a microorganism to an antimicrobial medicine to which it was previously sensitive. Origin of the antimicrobial resistance in bacteria occurs in two ways; it can be natural resistance or acquired resistance (Reygaert et al., 2018). Natural resistance of bacteria aganist antimicrobials indicate the inherent capability of bacterial species to withstand the effects of antibiotics, and this resistance is generated due to their genetic makeup, cellular structure, and metabolic pathway (Livermore et al., 2012). Acquired resistance of bacteria against antimicrobial components gained by the genetic mutation, genetic material transfer, and selective pressure, and it also can occur from inaccurate diagnosis, inappropriate prescription of antibiotics, and inadequate and overuse of antibiotics (Uddin et al., 2021). A major driver of antimicrobial resistance (AMR) in animals is overuse of

antibiotics for disease prevention and growth promotion and subtherapeutic doses of antibiotics are also responsible for the growth of resistant strains (Boeckel et al., 2015). A recent study showed that 80% of the livestock received antibiotics at certain stages for treating infections (Van et al., 2020). Only 10% of antibiotics for treatment of infection and most of the parts are used for enhancing growth and prevention of disease (Fabio et al., 2019). Use of the antibiotic in food-producing animals is significantly responsible for the transmission of resistant bacteria (Caneschi et al., 2023). Antibiotic-resistant bacteria (ARG) and antibiotic residues can transfer into wild animals in various routes. Manure of the food-producing animals used in the land acts as a reservoir of resistant bacteria and antibiotic residues; these spread into the wild animal population (Libisch et al., 2020). Food-producing animals, especially poultry, may be an important source of antibiotic-resistant bacteria due to extensive use of antibiotics, including third-generation antibiotics, in industrial farming (Mark et al., 2014). Chicken meat can transfer antimicrobial resistance. It can horizontally transfer antibiotic-resistant genes through plasmids and transposons (Shousha et al., 2015). Antimicrobial-resistant genes containing bacteria from animals can be entered into the food chain and transferred to another animal by eating raw meat (Bennani et al., 2020). Raw meat-based diets of animals contain a risk due to being contaminated with antimicrobial-resistant bacteria. It is also recognized as a risk factor for shedding antimicrobial-resistant bacteria in animals. This is one of the most concerning threats to animal health (Nüesch et al., 2019). Antimicrobial resistance can be transmitted in tigers in captive conditions in different ways. In captive conditions, tigers remain in close contact with humans that can transfer antimicrobial resistance bacteria from humans to animals (Xue et al., 2016). In captive conditions, tigers required a high protein and fat diet (Bradi et al., 1994). Tigers in captivity fed raw meat diets consist of broiler meat, beef, whole poultry, or rabbit carcass (American Feed Industry Association., 2020). These types of feed ingredients can transfer antimicrobial resistance to tigers in captive conditions.

The Chattogram Zoo has 18 tigers of different ages and different sexes, situated in Chattogram district in Bangladesh. One of the tigers was injured due to fighting. The main objectives of this case study are:

1. To describe the susceptible and resistant antibiotics.

2. To describe the treatment procedure of the tiger that carries multiple drug-resistant bacteria.

Figure 1.1 Wound present in the shoulder region of the tiger

Chapter 2: Meterials and Methods

2.1.Case History and Clinical Examination

Beli is a 3.1-year-old female bangle tiger of the Chattogram Zoo. The total number of tigers in the zoo is 19, and they are kept in the cage. On 8 April 2024, a wound was detected on shoulder region of Beli, and it was assumed that this was caused by fighting. Previous year, this tiger was also affected due to fighting and recovered with the use of the antibiotic Ceftriaxone.

After this recent injury, the tiger was treated with the antibiotic flucloxacillin at 10 mg/kg body weight in an 8-hour interval for 13 days. But it did not respond to this treatment.

2.2.Laboratory Test

Sample collection, transportation and preservation: For the bacteriological culture and culture sensitivity test, a swab sample was collected from the wound of the tiger. A sterile cotton bud was gently rubbed on the wound area and then dissolved in 3 ml of normal saline that was contained in the falcon tube. The marked falcon tube was placed in an icebox that maintained 4°C temperature and carried to the Udder Health Bangladesh (UHB) lab and maintained 4°C temperature upto inoculation sample into agar.

Bacteriological Culture: Bacteriological culture was done for the isolation and identification of the bacteria from the sample collected from the tiger's wound. The collected sample was spread on blood agar medium and incubated for 24 hours at 37 °C.

Culture Sensivity Test: For the detection of the sensitive antibiotic in this tiger, it was done a culture sensitivity was performed. For the purpose of the culture sensitivity, a swab sample was collected manually from the affected area of the tiger without using any anesthetic. And the swab sample medium was kept in normal saline. The CS test was done by the Kirby-Bauer disc diffusion method (CLSI ., 2020).

2.3.Treatment Strategy :

First Phage: After the injury, the tiger was treated with the antibiotic flucloxacillin at 10 mg per kg body weight at 8-hour intervals via the intramuscular route for 13 days. But when it was not responsive, then bacteriological isolation and CS tests were done for the identification of sensitive antibiotics. In bacteriological culture, bacteria grew on blood agar. In MacConkey agar, produce slightly opaque growth and pink-colored colonies on the EMB agar were developed. This indicates the presence of gram-positive and gram-negative bacteria. CS test results showed that the antibiotics Clindamycin and Ceftriaxone were resistant and Cefazolin was sensitive. Then treated with Cefazolin (Ing. Zolibac-1 gm) at 20 mg per kg body weight in an 8-hour interval via the intramuscular route for 13 days.

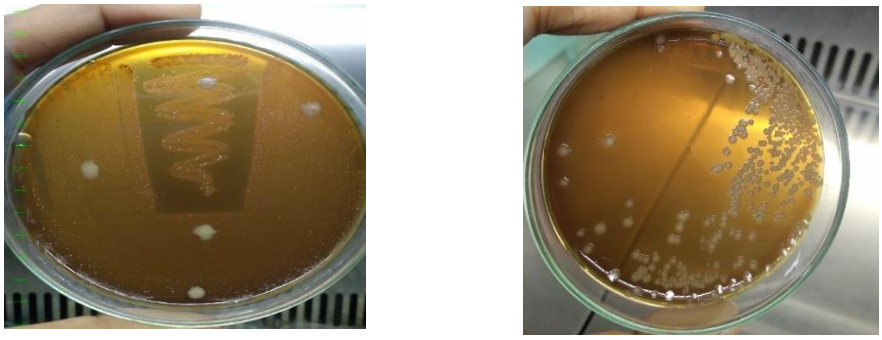

Figure 2.1. Large yellow colour colonies growth on the blood agar media

Second Phage: The tiger was treated with the antibiotic Cefazolin (20 mg/kg body weight) for 13 days, but this did not respond. Then bacteriological culture and CS tests were done repeatedly. In bacteriological culture, bacteria grow on blood agar but not in MacConkey agar and EMB agar, which indicate the only presence of gram-positive bacteria and the absence of gram-negative bacteria. The CS test result showed that the sensitive antibiotic was ciprofloxacin and the resistant antibiotics were amoxicillin+clavulanic acid, ceftriaxone, ciprofloxacin, gentamicin, cefotaxime, ceftazidime, and ampicillin. Then the antibiotic ciprofloxacin was provided at 15 mg per kg body weight intramuscularly in 8-hour intervals for 12 days.

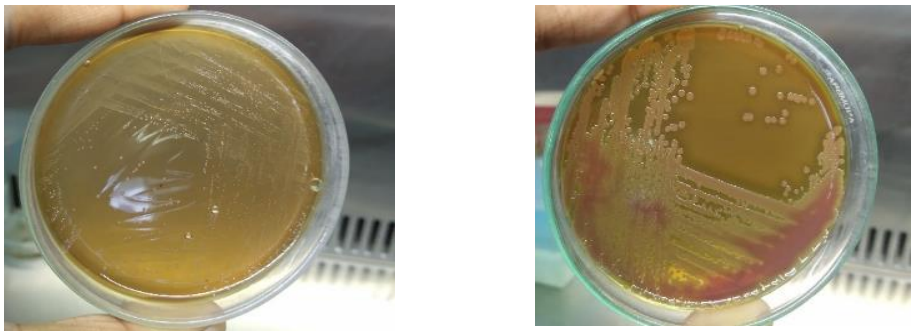

Figure 2.2. White colour colonies produced in the blood agar media

Third Phage: After use of the antibiotic ciprofloxacin at 15 mg per kg body weight for 12 days when it was not working, then bacteriological culture and CS test were done further. In bacteriological culture, bacteria grew on blood agar and produced white-colored colonies. And produced pink color colonies on the MacConkey agar and EMB agar. This result was indicative of the presence of gram-positive bacteria and gram-negative bacteria. And whitish colonies were grown on the mannitol salt agar that indicated the presence of coagulase-negative staphylococci. CS test results indicate that the antibiotics linezolid and vancomycin were sensitive, and the antibiotics ampicillin, cefoxitin, oxacillin, erythromycin, ciprofloxacin, amoxicillin + clavulanic acid, Penicillin, gentamicin, Sulfamethoxazole + Trimethoprim, Ceftriaxone and tetracycline were resistant. After these tests, the tiger was treated with the antibiotics linezolid (Tab. Linzolid-600 mg), using one tablet (600 mg) at 12-hour intervals orally for 15 days. After this medication, the tiger was fully recovered.

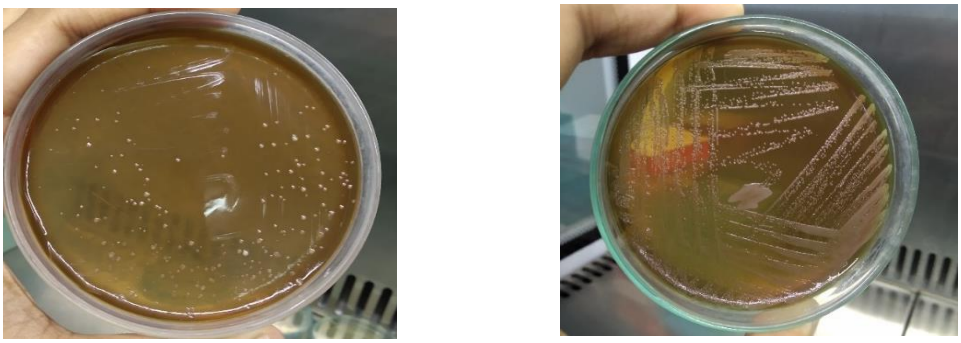

Figure 2.3. White and transparent colonies produced in the blood agar media.

Table 2.1 Culture sensitivity test (CS) of the tiger where sensitive antibiotics and resistant antibiotics are detected and treatment was given with sensitive antibiotics.

SS (RC) Date	**Sensitive antibiotics**	**Resistant antibiotics**	**Treatment**
27/04/24 (28/04/24)	Cefazolin, Vancomycin (intermediate)	Clindamycin, Ceftriaxone	Yes (28/04/24) (Inj.Zolibac)
13/05/24 (15/05/24)	Ciprofloxacin	Amoxycillin+clavulanic acid, Ceftriaxone, Ciprofloxacin, Gentamycin, Cefotaxime, Ceftazidime, Ampicillin	Yes (15/05/24) (Inj. Ciprocin)
13/05/24 (16/05/24)	No bacterial growth	-	No
23/05/24 (26/05/24)	All tested antibiotics are resistant	Ciprofloxacin, Ceftazidime Amoxycillin+clavulanic acid, Ceftriaxone, Ciprofloxacin, Gentamycin, Cefotaxime,	No
23/05/24 (26/05/24)	Linezolid, Vancomycin	Ampicillin, Cefoxitin, Erythromycin, Ciprofloxacin, Amoxycillin+clavulanic acid, Penicillin, Gentamycin,	Yes (26/05/24) (Tab Linezolid)

****SS= Sample submission date, **RC= Report collection date**

Chapter 3: Discussion

The current case report was considered to describe the antibacterials used to treat the injured tiger. After the injury, the tiger was treated with flucloxacillin. It is a narrow-spectrum, beta lactamase resistance penicillin (kennard et al., 2019). Flucloxacillin used at 10 mg per kg of body weight, with an 8-hour interval for 13 days on the intramuscular route. Recently, the use of the antibiotic flucloxacillin has increased for treatment purposes, and this overuse can develop antimicrobial resistance (Francis et al., 2016). After the use of the flucloxacillin, the tiger was not recovered, maybe due to antimicrobial-resistant bacteria.

When it didn't work, then the CS test was done, and it showed that the antibiotic Cefazolin was sensitive. Cefazolin is a first-generation antibiotic under the group of cephalosporins (Kirby et al., 1973). Then use Cefazolin 10 mg/kg body weight intramuscularly for 12 days but the wound was not recovered. In a study, cefazolin showed less effectiveness against some strains of Staphylococcus aureus (Weis et al., 2019). Methicillin-susceptible *Staphylococcus aureus* (MSSA) strains can produce type A beta-lactamase; these are responsible for the failure of cefazolin (Nannini et al., 2019).

Further CS tests identified Ciprofloxacin as the sensitive antibiotic. Ciprofloxacin at 15 mg/kg body weight at 8-hour intervals intramuscularly was used for 12 days. Ciprofloxacin is an antibiotic under the group of fluoroquinolones, and it is effective against gram-positive and gram-negative bacteria and also against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* (Terp et al., 1987). But it was not worked at all. Further CS tests showed the antibiotic ciprofloxacin was resistant but that was sensitive previously. Due to improper use of this antibiotic, it might have developed resistance (Habboush et al., 2018)

At the last CS test, the result indicated the sensitive antibiotic was linezolid. Linezolid is a synthetic antibiotic under the class of oxazolidinones. It is highly effective against gram-positive bacteria and indicated for the treatment of skin layer infection (Azzouz et al., 2024). Linezolid is highly sensitive to the methicillin-resistant *Staphylococcus aureus* or *Streptococcus pyogenes* that are responsible for the skin infection. It is also effective against vancomycin-resistant enterococcus

(Hasemian et al., 2018). Then use linezolid as the dose of 600 mg at 12-hour intervals for 15 days. After this treatment the tiger was recovered.

Figure 3.1. Recovered the wound of the tiger after using the antibiotic linezolid

Chapter 4: Conclusion

Antimicrobial resistance (AMR) has become a worldwide threat to animal and public health. Feedstuff can play a role in transferring antimicrobial resistance genes and antimicrobial-resistant bacteria, which can grow antimicrobial resistance in animals. Raw meat can act as a vector for transmission of antimicrobials resistance. Poultry and livestock act as a reservoir of the antimicrobial-resistant organisms. Excessive use of antibiotics for increasing production and treatment of disease in the poultry and livestock sector is responsible for this. These types of raw meat can serve as new drug-resistant and multidrug-resistant bacteria through the food chain. Antimicrobial resistance was developed in this tiger due to feeding on the raw meat and misuse or inappropriate doses of antibiotics.

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Biography

This is Anik Dutta from Chattogram, son of Rupandra Lal Dutta and Shika Dutta, I have completed my Secondary School Certificate examination in 2016 (GPA: 5.00) from Paraikora Nayantara High School, Anowara, Chattogram followed by Higher Secondary Certificate examination in 2018 (GPA: 4.50) from Patiya Government College, Patia, Chattogram. Then, I enrolled for Doctor of Veterinary Medicine (DVM) degree in Chattogram Veterinary and Animal Sciences University, Chattogram, Banglaesh in 2018-2019 session. Now, I am entrolled in the year-long internship program under Faculty of Veterinary Medicine, CVASU. In future, I would like to work in field as a veterinary doctor.