

# Isolation of *Citrobacter freundii* from a dermal lesion of a captive Green iguana (*Iguana iguana*)

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## ABSTRACT

A 1-year-old Green iguana (*Iguana iguana*) was brought to the clinic with a complaint of a slowly growing mass on the left mandible and symptoms of weakness and loss of appetite for several weeks. The clinical examination noted that the mass was quite firm, invasive to the region, and had a necrotic appearance. It was removed by surgical operation and sent to the laboratory for investigation. In the microbiological evaluation, *Citrobacter freundii* was isolated and identified very intensely. According to the laboratory results, enrofloxacin was chosen as the broad-spectrum antibiotic and used for 1 week.

The reptilian class, which includes many animal groups such as turtles, lizards, iguanas, and snakes, differs from the common pet animals in terms of microbiota. In the modern world, the feeding of these animals in homes as pets has increased the risk of many diseases not only in themselves but also in humans. They are considered to be the source of many gram-positive and negative bacterial infections that can be transmitted especially through urine saliva, blood, wounds and breathing to humans. In particular, immunocompromised individuals and children are in the risk group (2). *Citrobacter freundii* is a species of facultative anaerobic Gram-negative bacteria of the family Enterobacteriaceae. It can be isolated from reptiles: tortoises, iguanas, lizards, chelonians, snakes (1, 3, 8). Considering that the bacterium is zoonotic, this resistance can also pose a serious danger to humans (6). In this case report, it has been revealed the presence of an opportunistic bacteria found in normal flora of a captive

iguana skin lesion and successful treatment was performed. It has been underlined that unsuitable environmental conditions (temperature, humidity) may be effective in the disease.

A one-year-old, female green iguana (*Iguana iguana*) was brought to the clinic with complaints of appetite and lethargy. On clinical examination, a solid, necrotic mass was detected in her left jaw (Figure 1). According to the anamnesis, it was learned that the animal had a terrarium, but spent most of its time at home. Medetomidine HCl (0.1 mg/kg) was administered intramuscularly for preanesthesia. After 20 minutes, general anesthesia was induced with ketamine HCl (5 mg/kg, intramuscular). Approximately 30 minutes after ketamine injection, iguana was intubated and anesthesia was maintained with Isoflurane (100% oxygen) (Figure 1). The mass was removed totally and the wound was sutured with 4/0 non-absorbable monofilament suture



**Figure 1.** A solid, necrotic mass on the green iguana's left jaw.

material. The material was sent to the laboratory as soon as it is taken. Carprofen (2 mg/kg, intramuscular) was administered for 3 days for analgesia.

Inoculations were made on blood agar and MacConkey agar for bacterial identification. After incubation under aerobic and microaerobic conditions, hemolyzed colonies grew on blood agar and lactose-fermenting colonies on MacConkey agar. Gram-negative rod-shaped bacteria were detected after Gram staining of the colonies. According to biochemical tests, the causative agent was determined to be *Citrobacter freundii*. Confirmation of the isolated and identified strain was performed by MALDI-TOF-MS (Matrix-assisted laser desorption/ionization-time of flight mass spectrometry).

Antibiotic susceptibility testing of the isolate was performed according to the Kirby-Bauer disc diffusion method recommended by NCCLS (NCCLS, 2013). For this purpose, gentamicin (10 ug), oxytetracycline (30 ug), enrofloxacin (5 ug), amoxicillin (10 ug), chloramphenicol (30 ug), tetracycline (30 ug), erythromycin (15 ug), sulphamethoxazole/trimethoprim (1.25 µg/23.75 µg), lincomycin (2 µg) and ceftiofur (30 µg) containing commercial antibiotic discs were used. A small number of colonies taken by the inoculation loop was passed into Nutrient Broth (NB) and distributed homogeneously. NB's were incubated at 37 °C for 24 hours and adjusted to McFarland 0.5 (108 microorganisms/ml) after turbidity. The sample taken from that suspension with the help of a sterile swab was inoculated onto the Mueller-Hinton agar surface. Subsequently, discs containing different antibiotics were placed on the agar surface with the help of sterile forceps and incubated at 37 °C for 24 hours. Inhibition zones formed after incubation were measured.

After the measurement, it was determined that the agent was sensitive to gentamicin, oxytetracycline, enrofloxacin, chloramphenicol, tetracycline, sulphamethoxazole /trimethoprim and resistant to other antibiotics. According to the laboratory results, enrofloxacin was chosen as the broad-spectrum antibiotic and given orally for 1 week after the operation.

The choanae, cloacae and conjunctival bacterial flora of healthy captive green iguanas may consist of *Acinetobacter spp.*, *Stenotrophomonas maltophilia*, *Pseudomonas spp.*, *Citrobacter spp.*, *Proteus vulgaris*, *Enterobacter spp.*, *Serratia spp.*, and *Salmonella spp.* These are the opportunistic pathogens in iguanas and many reptiles (8, 9). *Citrobacter spp.* is associated with ulcerative skin diseases, stomatitis, cloacitis, abscesses and respiratory infections (4). *Citrobacter freundii* was identified in the lesion of this case. There has been no report that characterized opportunistic bacteria in the gastrointestinal flora of green iguanas cause disease alone.

Poor husbandry may result in pathogen infections such as *Citrobacter spp.* in reptiles. In cases where terrarium cleaning is done adequately, soil and water create a suitable environment for the proliferation of such bacteria. Adverse conditions caused disease in reptiles, as well as horizontal transmission (5). Since the green iguana in this case report spends most of its time outside the terrarium, it causes contamination of the same environment that it shares with its owner. Conditions, where environmental hygiene is not provided adequately, can create a contaminated environment, such as the soil effect in the terrarium. This kind of condition will increase the exposure of the iguana to an opportunistic pathogen and reduce its antimicrobial activity severely.

Temperature plays an important role in reptiles' bacterial activity. Poor thermal acclimation changes their blood antimicrobial activity and disrupts the immune system, behavioural and physiological features. Also, reptiles increase body temperature in response to an infection like mammals; however, because they are ectotherms, they try to use warmer environmental conditions to raise their body temperature (10, 11). The fact that the green iguana reported in this case does not spend its time in the terrarium means exposure to the heat of the home environment. This unfamiliar condition affects the immune system of the green iguana after a while and increases the ability of opportunistic microorganisms to cause disease. However, the pathology showed up as a skin lesion in this animal.

Many bacterial isolates are potential zoonoses such as *Citrobacter spp.* *Citrobacter freundii* is a turtle pathogen and can also develop many bacterial zoonoses in immunosuppressed turtles. Especially children, immunosuppressed, elderly or debilitated people are at high risk for reptile-derived *Citrobacter spp.* *Citrobacter freundii* can cause infections such as urinary tract infections, diarrhoea, pneumonia or meningitis in humans (5).

The treatment is provided by choosing the appropriate antibiotic according to the antibiogram test results. However, due to the widespread use of broad-spectrum antibiotics, *Citrobacter freundii* has become increasingly resistant (7). In this case report, the antibiotic was selected according to the antibiogram test results. It was noted that there was a rapid improvement in the general condition of the patient after the operation.

In conclusion, little information is available regarding bacterial diseases in green iguanas. They have an adaptive immune response. However, climate and environmental changes may cause complete disruption of the function of bacterial flora. Additional research is necessary to determine the effects of normal flora on infectious diseases in iguanas. Until then, we have clear data on dangerous zoonotic agents for humans. Preventive medicine is vital. Veterinarians should inform owners of the risks of hygienic conditions for reptiles.

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### Conflict of Interest

The authors declared that there is no conflict of interest.

### Author Contributions

BB, KGC and IE conceived and planned the report. IE took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

### Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

### Ethical Statement

This study does not present any ethical concerns.

### Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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