

PREVALENCE OF *CITROBACTER* SPP. FROM PET TURTLES AND THEIR ENVIRONMENT



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Abstract

Pet turtles are considered a source of bacterial infection to humans when handled in captivity. Turtles purchased from 9 pet shops and 8 online markets in Korea were examined to determine whether the turtles and their environment were contaminated with *Citrobacter* spp. Biochemical tests and morphology revealed that *Citrobacter* spp. were isolated from 7 fecal and 76 environmental samples. Among the 7 fecal isolates, 5 bacteria were identified as *Citrobacter freundii* through 16S rRNA gene sequencing. The isolation rate of *Citrobacter* spp. from soil and water samples increased over time. Each of the isolate's antibiotic resistance was characterized with a disk diffusion test. The strains showed susceptibility against amikacin, ceftriaxone, ciprofloxacin, imipenem, sulfamethoxazole/trimethoprim, and tetracycline, but were resistant to cefoxitin, cephalotin, and chloramphenicol. These results indicate that pet turtles are a potential source of *Citrobacter* infection in humans in Korea. Copyright 2017 Elsevier Inc. All rights reserved.

Key words: prevalence; *Citrobacter* spp.; pet turtles; *Citrobacter* spp. infection; antibiotic resistance

Citrobacter, a genus from the Enterobacteriaceae family, are Gram-negative, facultative anaerobic bacteria that appear as coccobacilli or rods.¹ *Citrobacter* spp. are motile and use their peritrichous flagella to move.² *Citrobacter* spp. ferment mannitol with production of H₂S, and can use citrate as their sole source of carbon.^{2,3} *Citrobacter* spp. are rare opportunistic nosocomial bacteria that can cause urinary tract, hematologic, or neonatal infections (e.g., meningitis, sepsis, joint, general bacteremia); intraabdominal sepsis; brain abscesses; or pneumonia.⁴ *Citrobacter* spp. infections can be fatal, with 33% to 48% overall death rates being reported including 30% for children.^{3,5} Children and immunocompromised, elderly, or debilitated patients are at increased risk of infection.^{3,6} *Citrobacter* spp. are prevalent worldwide, as they are a component of the normal intestinal flora of humans.⁷ Hosts of *Citrobacter* spp. include humans, animals, and aquatic species (e.g., catfish, rainbow trout, and turtles).^{2,8-11} *Citrobacter* spp. are also abundant within various environments (e.g., water, soil, and food) because of fecal contamination. Among *Citrobacter* spp., *C. freundii* is frequently associated with reptiles, particularly as a turtle pathogen. The infection caused by *C. freundii* in animals is often related to an immunocompromised state initiated and exacerbated by poor husbandry conditions and other stress factors.¹¹ *Citrobacter* spp. can cause septicemic cutaneous ulcerative disease (SCUD) in turtles, and is characterized by distinctive clinical signs (e.g., anorexia, lethargy, liver necrosis, and petechiae) on the carapace and skin. The scutes are often damaged and slough, and an underlying discharge of purulent material may be present.¹² In the highly intensive production practices found on turtle farms, the disease can be transmitted to all uninfected turtles within 1 week.¹¹ Currently, on a global basis, pet reptile rearing has become increasingly popular. Reptiles are raised in approximately 453,000 households in the USA.¹³ Among them, pet turtles are the most popular for their small size and appearance.¹³ However, reptiles carry many typical enteric bacteria that have been classically associated with warm-blooded animals (i.e., salmonellosis has been acquired through contact

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with pet turtles).^{14,15} Moreover, *Citrobacter* spp. can infect humans if the owner fails to handle a pet turtle with care. The bacteria can be transmitted by physical contact with the infected turtles or through contaminated environments such as water and soil in the animal's cage. The U.S. Food and Drug Administration (FDA) has banned the sale of small turtles with a carapace length <4 inches to prevent the transmission of pathogenic bacteria from turtles to small children¹⁶; however, there are no such regulations in Korea. To prevent *Citrobacter* spp. infection by pet turtles, it is necessary to maintain sanitary and hygienic management of pet turtles and their environment. Though several antibiotics for treatment of *Citrobacter* infections are known, they are not always effective.¹⁷ Furthermore, repeated use of antibiotics may lead to multiple antimicrobial resistance of *Citrobacter* spp. organisms.¹⁸ The objectives of this research investigation were to determine the prevalence rate of *Citrobacter* spp. cultured from fecal samples of pet turtles purchased in pet shops and online markets, and of the animal's environment (soil) over time. The authors also measured resistance of the *Citrobacter* spp. isolates against several common antimicrobial drugs and characterized the cultured bacteria. The results of this study were used to assess the risk of infection from pet turtles and their environment from a public health standpoint and provide information concerning the prevention of *Citrobacter* spp. infection to humans exposed to reptiles.

MATERIALS AND METHODS

Purchase of Pet Turtles

A total of 34 turtles of 6 commercially popular species were purchased through 9 pet shops and 8 online markets in Korea. The randomly purchased turtles had an average weight of 15 ± 2 g, carapace diameter of 40 ± 5 mm, and were less than 4 weeks of age. All the tested turtles were determined to be healthy based on an external physical examination and did not have any clinical signs of SCUD. Among the 34 turtles, 10 Chinese stripe-necked turtles (*Ocadia sinensis*), 8 yellow belly sliders (*Trachemys scripta scripta*), 6 river cooters (*Pseudemys concinna concinna*), 4 northern Chinese softshell turtles (*Pelodiscus maackii*), 3 western painted turtles (*Chrysemys picta belli*), and 3 common musk turtles (*Sternotherus odoratus*) were evaluated.

Fecal Sample Collection

The fecal samples were collected immediately after the turtles were purchased. Each turtle was placed in a 500 mL beaker with 5 mL of sterilized distilled water for 24 hours. As a fecal sample, 1 mL of the distilled water containing the turtle's feces was collected. To prevent *Citrobacter* spp. transmission among individuals, poly-gloves were changed each time an animal was handled.

Enrichment and Isolation of *Citrobacter* spp.

The fecal samples were suspended in 3 mL of sterile saline, and 1 mL of the suspension was inoculated into 9 mL of tetrathionate broth (MBcell Ltd., Seoul, Korea) at 37°C for 24 hour.

Following the incubation procedure, the broth was mixed for 5 seconds with a Vortex agitator. Then one loopful from each tube was streaked onto a plate of MacConkey agar (MBcell. Ltd, Seoul, Korea) and incubated at 37°C for 24 hours. Pinkish colonies with a glossy surface were considered candidates for *Citrobacter* spp. isolates and were subcultured onto a plate of eosin methylene blue agar (EMB; MBcell. Ltd, Seoul, Korea) and incubated at 37°C for 24 hours.

Environmental Conditions of Pet Turtles in Cages

A total of 17 cages, each containing 1 to 3 turtles of the same species from the same pet shop, were used for the study; each cage had a slope made with soil and pebbles, 2 L of sterilized water, and a canister filter to maintain water quality. Feeding of turtles followed general husbandry methods.¹⁹ Gammarus dried shrimp with calcium supplements (Samhotech Co., Ltd., Seoul, Korea) were fed 3 times a day and vitamin D₃ powder (Rep-Cal Research Labs, Los Gatos, CA USA) was mixed with the feed every 5 days. Water temperature and pH were maintained between 26 ± 2 °C and 6.5 to 8.2, respectfully, and 12 hours of photoperiod was provided each day to the animals during the research investigation.

Isolation Rate of *Citrobacter* spp. from Environment Over Time

To determine the change in *Citrobacter* spp. infiltration of the turtle's environments over time, water and soil samples were collected every 2 days from each of the 17 cages for 10 days. Using a

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