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Isolation and Characterization of *Morganella morganii* from Asian Water Monitor *Varanus salvator*

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Abstract : An Asian water monitor *Varanus salvator* with physical wound due to bite which was subsequently infected with bacterium resulting to hemorrhage and pus in the skin blisters, abdominal distention and septicemia. *Morganella morganii* was isolated and identified from the blood and kidney of the reptile, and confirmed by PCR and biochemical tests. The sensitivity of isolated strains to different groups of antibiotics was also evaluated using the disc diffusion method. Pathogenicity test using *M. morganii* (SNUFPC-MM01) (1.6×10^{11} CFU/mouse) to suckling and adult mice resulted to the death of all mice. This paper describes the first isolation of *M. morganii* from Asian water monitor in Korea.

Key words **:** *Morganella morganii,* Asian water monitor, antibiotics, pathogenicity test.

Introduction

Morganella morganii is a gram-negative rod and facultative anaerobic bacteria; formerly, this organism is known as *Proteus morganii* and was classed in the genus *Proteus* (10,20). The genus is one member of the *Proteeae* tribe that includes *Proteus* and *Providencia*. It consists of one species, *M. morganii*, with two subspecies, *morganii* and *sibonii* (2), depending on the ability to utilize trehalose (8). *M. morganii* strains that ferment trehalose were designated as *M. morganii* ssp. *sibonii* and those that are not able to utilize this carbohydrate were designated as *M. morganii* ssp. *morganii* (8,19). The Asian water monitor *Varanus salvator* is the second largest lizard species in the world and is widely used for commercial purposes (17). These lizards consume a wide variety of prey, including commensal vertebrates (e.g. rats, chickens) and invertebrates (e.g. insects, crabs) (18).

Case

An Asian water monitor was presented for postmortem examination. The Asian water monitor (total length 211 cm, weighting 17.8 kg) had come from one of the private commercial aquarium in Seoul, South Korea that was reared for public exhibition. It was reported to have a physical wound in the left brachial area due to bite. The animal died due to symptoms such as anorexia, lethargy and depression persisting for two weeks. Shortly after death, its body was submitted to the College of Veterinary Medicine, Seoul National University for postmortem examination.

At necropsy, gross findings included hemorrhage and pus in the skin blisters as well as abdominal distension were observed. The tissues of the internal organs were observed to be necrotic and lytic, hemorrhagic and vesicular abscesses were scattered throughout the liver (Fig 1). Sterile swabs from kidney and blood samples were collected aseptically and were inoculated on to blood agar (Komed, Seoul, Korea) supplemented with 5% defibrinated sheep blood and incubated for 24 h at 25°C. After incubation, single colonies from plates with dense, virtually pure culture growth were re-streaked on the same media. The suspected colonies were collected and performed for Gram staining, motility, catalase and oxidase tests. It was identified through PCR assay and Vitek II system in order to confirm the level of subspecies. We used PCR primers which were previously developed for specific detection of *M. morganii* based on the 16S rDNA sequence (9). The result of biochemical test was compared to reference strain from Bergey's Manual of Determinative Bacteriology. Antimicrobial susceptibility test of the isolate was performed using 25 antimicrobial drugs (amikacin, ampicillin, amoxicillin/clavulanic acid, carbenicillin, cefepime, cefixime, cefoperazone, cefotaxime, chloramphenicol, ciprofloxacin, colistin, enrofloxacin, gentamicin, kanamycin, nalidixic acid, neomycin, nitrofurantoin, norfloxacin, ofloxacin, oxytetracyclin, polymyxin B, sulfamethoxazole/trimethoprim, tetracycline, tobramycin and trimethoprim) through the standard method used by Bauer *et al.* (1) on Muller Hinton agar (MHA; Difco, USA). Result of the identification revealed that the isolated bacterium was Gram negative, rod in shape and motile. Using *M.*

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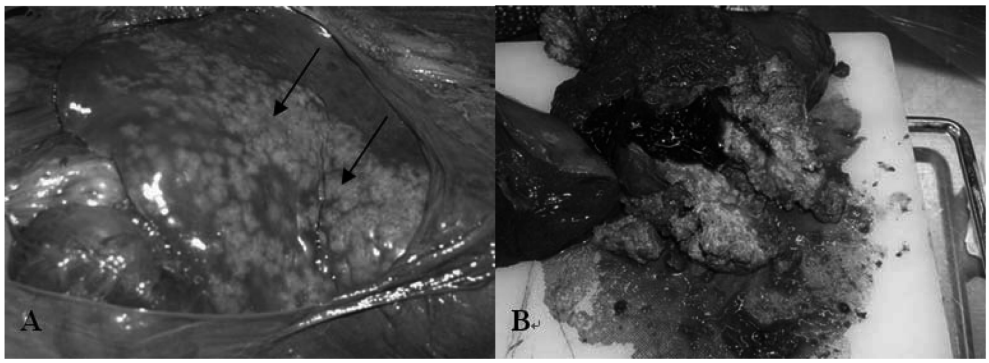

Fig 1. Necropsy in Asian water monitor. (A) Vesicular abscesses were scattered throughout the liver (arrow). (B) Internal organs were hemorrhagic liquefied necrosis.

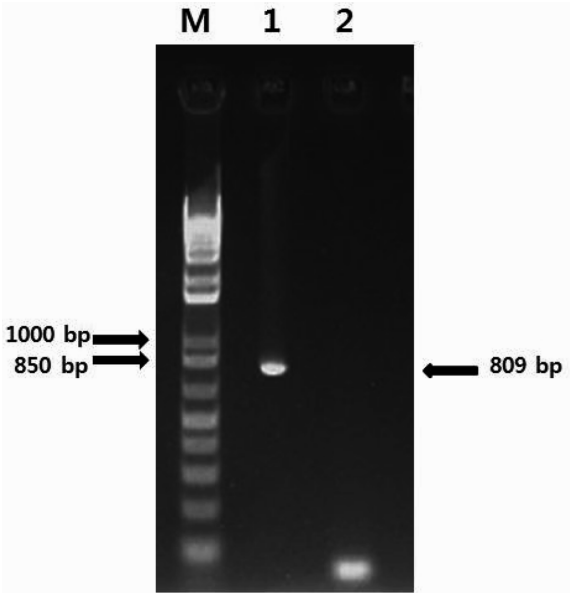

Fig 2. Amplification products obtained using the specific primer for detection of *Morganella morganii*. Lane M: 100 bp DNA ladder; Lane 1: PCR positive for *M. morganii* using specific primer (809bp); Lane 2: negative control.

morganii specific PCR primers, the present result revealed positive band at 809 bp (Fig 2) similar to the result previously reported (9). The isolate also showed a positive result on the catalase but negative in oxidase tests. Based on the results of PCR and biochemical tests, the strain was identified as *Morganella morganii*. Additionally, based on its inability to ferment trehalose, the isolated strain was further classified as *M. morganii* ssp. *morganii* (Table 1) (13). It was further characterized that the present isolate was lysine decarboxylase (LDC)-negative and ornithine decarboxylase (ODC)-positive (Table 2) in which the final biochemical identification was *M. morganii* ssp. *Morganii* Biovar A. The results of the susceptibility pattern in some antibiotics (Table 3) of *M. morganii* ssp. *morganii* (SNUFPC-MM01) isolate of the present study were almost similar to the results of Penner *et al.* (15) regarding antibiotics investigation of the *Morganella* strain.

Table 1. Trehalose test for differentiation of *M. morganii* subspecies

M. morganii subspecies	Trehalose
M. morganii ssp. *morganii*	–
M. morganii ssp. *sibonii*	+
SNUFPC-MM01	–

Table 2. LDC and ODC tests for differentiation of *M. morganii* ssp. *morganii* Biovar type

Biovar type	LDC	ODC
M. morganii subsp morganii Biovar A	-	+
M. morganii subsp morganii Biovar B	+	+
M. morganii subsp morganii Biovar C	-	-
M. morganii subsp morganii Biovar D	+	-
SNUFPC-MM01	-	+

SNUFPC-MM01 isolate was resistant to nalidixic acid, chloramphenicol, and aminoglycosides but not to tobramycin and gentamicin which was the opposite result previously reported (15). In order to determine whether the SNUFPC-MM01 isolate was pathogenic to mammals or not, the isolate was intraperitoneally injected with a concentration of 1.6×10^{11} CFU/mouse to five suckling and adult mice. All mice died after 48 hours. Upon necropsy, hemorrhage and necrosis were observed in the kidney and liver (Fig 3). Re-isolation and identification of the bacteria from the kidney and blood were done using culture and Vitek II system in order to fulfill the Kock's postulates and the results showed the same bacteria (data not shown).

Discussion

Like other *Enterobacteriaceae*, *M. morganii* is found in the environment and in the intestinal tract and feces of human beings, mammals, and reptiles as component of the normal microflora (21). It was frequently encountered in postoperative and immunocompromised humans, other mammals, avians, and

Table 3. Antibiotics susceptibility test for *M. morganii* ssp. *morganii* isolates

Antibiotics (ug)	Strain
	SNUFPC-MM01
Amikacin (30)	+
Ampicillin (10)	+
Amoxicillin/Clavulanic acid (30)	−
Carbenicillin (100)	++
Cefepime (30)	+
Cefixime (5)	−
Cefoperazone (75)	+
Cefotaxime (30)	+
Chloramphenicol (30)	+
Ciprofloxacin (5)	++
Colistin (20)	+
Enrofloxacin (5)	+
Gentamicin (10)	++
Kanamycin (30)	−
Nalidixic acid (30)	−
Neomycin (30)	+
Nitrofurantoin (300)	+
Norfloxacin (10)	+
Ofloxacin (5)	+
Oxytetracyclin (30)	−
Polymyxin B (300IU)	+
Sulfamethoxazole(23.75)/Trimethoprim (1.25)	−
Tetracycline (30)	+
Tobramycin (10)	++
Trimethoprim (5)	−

− (0 mm), + (1~10 mm), ++ (10~20 mm).

reptiles (5,14). When the host immunity was decreased, it becomes an opportunistic secondary invader and can be isolated from the blood, respiratory tract, wounds, and urinary tract in humans (7) that causes sepsis, pneumonia, urinary tract infections, wound infections, musculoskeletal infections, central nervous infections, pericarditis, chorioamnionitis, empyema, and spontaneous bacterial peritonitis (4,13). In animals, it has been isolated from the tissues of broiler chickens and considered as the possible cause of swollen head syndrome or respiratory disease (11,20). Lung lesions in animals associated with this bacterium have been described in a jaguar (3). *M. morganii* infections in reptiles has been reported in cases of American alligators with septicemia (12), and severe suppurative polyarthritis, secondary bacteremia in a West African dwarf crocodile (6). Generally, *M. morganii* causes disease in sites previously infected by other organisms, and may cause pyogenic infection if it is accidentally introduced into the body (16).

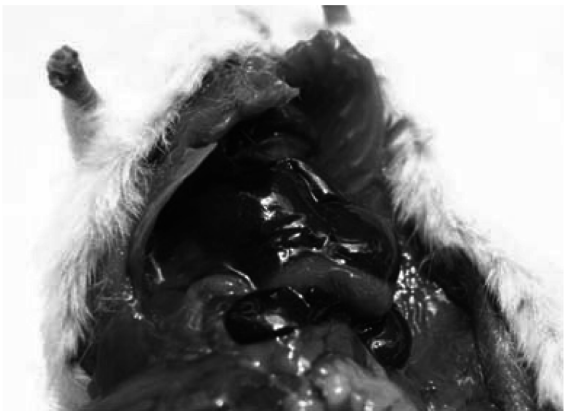

Fig 3. Necropsy in adult mouse. Liver showed a hemorrhagic necrosis and discoloration.

So far, the isolation of *M. morganii* has been reported in many cases from reptiles in other countries but not yet in Korea. In the present case, we identified *M. morganii* ssp. *morganii* Biovar A by PCR and biochemical tests. This report describes the first isolation of *M. morganii* from a large lizard known as Asian water monitor *Varanus salvator* in Korea. A wound infection due to bite was observed in which the *M. morganii* as an opportunistic secondary agent had entered and possibly cause septicemia to the animal. The isolation of *M. morganii* in kidney and blood in the animal as pure culture indicates that the bacterium was present in large numbers and was the possible primary cause of septicemia. The possibility that the animal was immunosuppressed could not also be excluded.

The results of antibiotic susceptibility test of the present study had slight difference from previously reported results of Penner *et al.* (15). We could consider two possibilities about these results. First, there was variation among the strains in susceptibility to antibiotics. Second, acquired resistance due to abuse and misuse of antibiotics, because the private commercial aquarium that submitted this sample used various antibiotics in treating their animals. Additionally, based on the results of the pathogenicity test on mice, this case documents the pathogenic potential and also a potential risk for transmitting *M. morganii* to immunocompromised individual include human.

Acknowledgment

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References

1. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method.

Am J Clin Pathol 1966; 45: 493-496.
2. Brenner DJ, Farmer JJ, Fanning GR, Steigerwalt AG, Klykken P, Wathen HG, Hickman FW, Ewing WH. Deoxyribonucleic acid relatedness of *Proteus* and *Providencia* species. Int J Syst Bacteriol 1993; 28: 269-282.
3. Choi JH, Yoo HS, Park, JY, Kim YK, Kim E, Kim DY. Morganelliasis pneumonia in a captive jaguar. J Wildl Dis 2002; 38: 199-201.
4. Cunningham ET Jr., Whitcher JP, Kim RY. *Morganella morganii* postoperative endophthalmitis. Br J Ophthalmol 1997; 81: 170-171.
5. Gebhart-Mueller Y, Mueller P, Nixon B. Unusual case of postoperative infection caused by *Morganella morganii*. J Foot Ankle Surg 1998; 37: 145-147.
6. Heard DJ, Jacobson ER, Clemmons RE, Campbell GA. Bacteremia and septic arthritis in a West African dwarf crocodile. J Am Vet Med Assoc 1998; 192: 1453-1454.
7. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. Bergey's manual of determinative bacteriology. 9th ed. Baltimore: Williams and Wilkins Co. 1994: 183.
8. Jensen KT, Frederiksen W, Hickman-Brenner FW, Steigerwalt AG, Riddle CF, Brenner DJ. Recognition of *Morganella* subspecies, with proposal of *Morganella morganii* subsp. *morganii* subsp. nov. and *Morganella morganii* subsp. *sibonii* subsp. nov. Int J Syst Bacteriol 1992; 42: 613-620.
9. Kim SH, An H, Wei CI, Visessanguan W, Benjakul S, Morrissey MT, Su YC, Pitta TP. Molecular Detection of a Histamine Former, *Morganella morganii*, in Albacore, Mackerel, Sardine, and a Processing Plant. J Food Sci 2003; 68(2): 453-457.
10. Koneman EW, Allen SD, Janda WM, Winn WC Jr. The Enterobacteriaceae. *In*: Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC Jr. Color Atlas and Textbook of Diagnostic Microbiology. 5th ed. Philadelphia: Lippincott-Raven. 1997; 213.
11. Lin MY, Cheng MC, Huang KJ, Tsai WC. Classification, pathogenicity, and drug susceptibility of hemolytic gram-negative bacteria isolated from sick or dead chickens. Avian Dis 1993; 37(1): 6-9.
12. Novak SS, Seigel RA. Gram-negative septicaemia in American alligators (*Alligator mississippiensis*). J Wildl Dis 1986; 22(4): 484-487.
13. O'Hara CM, Brenner FW, Miller JM. Classification, identification, and clinical significance of *Proteus, Providencia*, and *Morganella*. Clin Microbiol Rev 2000; 13: 534-546.
14. Okumoto M, Smolin G, Belfort R Jr, Kim J, Sivario CE. *Proteus* species isolated from human eyes. Am J Ophthalmol 1976; 81: 495-500.
15. Penner JL. Genus XIII *Morganella In*: Krieg NR, Holt JG. Bergey's Manual of Systematic Bacteriology. Baltimore: Williams and Wilkins. 1984; 497.
16. Roels S, Wattiau P, Fretin D, Butaye P, Vanopdenbosch E. Isolation of *Morganella morganii* from a domestic rabbit with bronchopneumonia. Vet Rec 2007; 161: 530-531.
17. Shine R, Harlow PS, Keogh JS, Boeadi. Commercial harvesting of giant lizards: The biology of water monitors *Varanus salvator* in southern Sumatra. Biol Conserv 1996; 77(2-3): 125-134.
18. Shine R, Ambariyanto, Harlow PS, Mumpuni. Ecological traits of commercially harvested water monitors, *Varanus salvator*, in northern Sumatra. Wildlife Res 1998; 25(4): 437-447.
19. Stock I, Wiedemann B. Identification and natural antibiotic susceptibility of *Morganella morganii.* Diagn Microbiol Infect Dis 1998; 30(3): 153-165.
20. Tanaka M, Takuma H, Kokumai N, Oishi E, Obi T, Hiramatsu K, Shimizu Y. Turkey rhinotracheitis virus isolated from broiler chicken with swollen head syndrome in Japan. J Vet Med Sci 1995; 57(5): 939-941.
21. Quinn PJ, Carter ME, Markey B, Carter GR. *Enterobacteriaceae*. *In* Quinn PJ, Carter ME, Markey B, Carter GR. Clinical Veterinary Microbiology. Madrid: Wolfe Publishing Co. 1994; 209-236.

아시아 물왕도마뱀에서 분리된 모가넬라 모가니의 분리동정

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요 약 : 왼쪽 앞다리에 교상을 입은 아시아 물왕도마뱀은 세균감염에 의해 피부 출혈, 농포, 수포, 복부팽만, 패혈증의 증상을 보인 후 폐사하였다. 신장에서 균을 분리, 생화학적 검사와 PCR 방법을 이용 모가넬라 모가니로 동정하였다. 25종의 항생제 감수성 검사를 실시하였고 분리된 균을 1.6×10^{11} CFU/mouse 농도로 마우스 복강에 접종, 포유류에 대한 병원성을 검사하였다. 실험결과 마우스는 모두 폐사하였다.

주요어 : 모가넬라 모가니, 아시아 물왕도마뱀, 항생제, 병원성 검사.