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CASE REPORT

Chromobacterium violaceum isolated from pleural effusion in cat and antimicrobial susceptibility profile: A rare case report

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ABSTRACT

Objective: This study aims to present *Chromobacterium violaceum* isolated from a pleural effusion in an indoor-outdoor cat.
Materials and Methods: A 3-year-old male domestic shorthair cat was brought to the hospital with dyspnea. The thoracic radiographs and ultrasound showed the presence of a pleural effusion. The thoracic fluid was sent for cytological analysis, reverse transcription polymerase chain reaction (RT-PCR) for feline infectious peritonitis (FIP), and bacterial culture.
Results: Cytological findings illustrated the protein-rich modified transudate. Albumin: globulin ratio was 0.4. RT-PCR testing for FIP was negative. The bacterial culture presented a pure growth of violet-pigmented colonies on both sheep blood agar and MacConkey agar. The isolation was identified and further confirmed as *C. violaceum*. The isolation was susceptible to drugs in aminoglycosides, tetracyclines, macrolides, fluoroquinolones, and sulfonamide groups.
Conclusion: *Chromobacterium violaceum*, a saprophytic Gram-negative bacterium, commonly inhabits stagnant water and soil in tropical and subtropical areas. It is considered an opportunistic bacterium in both veterinary and human medicine. Although *C. violaceum* infection is rare, the disease is extremely fatal. In Thailand, a few cases of humans infected with *C. violaceum* were reported; however, the bacterial infection has never been discovered in animals. This case report highlighted a rare opportunistic infection of *C. violaceum*in a cat in Thailand and suggested drugs of choice for clinical treatments.

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Introduction

Chromobacterium violaceum is a saprophytic Gram-negative rod-shaped bacterium that produces a brilliant violet pigment called violacein on both blood and MacConkey agar. It is normally found in soil and stagnant water in subtropical and tropical areas. Although infection by this organism is rare, it has caused mortality in both humans and animals. The first human case was reported in Malaysia by Sneath et al. [1]. In Thailand, a few cases of human infection were reported. Sirinavin et al. [2] reported an invasive *C. violaceum* infection in a 3.3-year-old child with abscesses in the lungs, liver, and spleen. Jitmuang [3] reported two cases of sepsis infection with fatal outcomes. *Chromobacterium violaceum* primarily enters the body through opened wounds [4,5]. It is also relatively common to get an infection by accidentally coming into contact with contaminated water or dust [6–9], either through the mouth or by inhalation. Papakhee et al. [10] reported *C. violaceam* isolated from a wound of a patient with an open fracture of the right forearm. Moreover, Roberts et al. [11] reported a patient who developed the infection after he had traveled to Thailand and cut his leg on the coral.

In animals, the potential pathogenicity of *C. violaceum* was first reported in three carabaos (*Bubalus bubalis carabanesis*) in the Philippines [12]. Furthermore, *C. violaceum* infection with serious outcomes was previously described

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in various species of animals, for example, red panda [13], calves [14,15], horses [16], dogs [17], swine [18], non-human primates [19], and cougars [20]. Particularly in cats, the previous report of *C. violaceum* infection demonstrated *C. violaceum* isolated from non-healing dog bite wounds [21]. Nevertheless, *C. violaceum* causing septicemia in cats has not been recognized before. Although no evidence of transmission between humans and animals has been reported, veterinarians should take special care in dealing with animals with a history of environmental exposure and broken skin. Moreover, the antimicrobial susceptibility profiles of saprophytic bacteria revealed the resistance to several antibiotics, including a last-resort antibiotic for humans. This should be alarming about the circulation of antimicrobial resistance genes in the environment. To the best of the author's knowledge, this report presents the first isolation of *C. violaceum* from a feline pleural effusion and its antimicrobial susceptibility profile.

Materials and Methods

A 3.4 kg, 3-year-old, male, domestic shorthair cat was present at the emergency room, Kasetsart University Veterinary Teaching Hospital, Kamphaeng Saen Campus, with a history of depression, decreased appetite, and dyspnea. According to history, the symptoms persisted for 2 days before the admission. The cat had been vaccinated for the feline panleukopenia virus, and no accident was noted. The physical examination revealed mild hyperthermia (102.9° F) and approximately 5% dehydration with a capillary refill time of less than 2 sec, pink mucous membrane, and dulled left lung sound.

The following diagnostic tests were performed: thoracic radiography, ultrasonography, complete blood count (CBC), and serum biochemistry. The thoracic radiographs revealed increased dense opacity of the left lung and the presence of a pleural effusion (Fig. 1A). The opacity obscured the left heart border and left hemidiaphragm. The ultrasounds confirmed the presence of a pleural effusion with hyperechoic sediments. The thoracocentesis yielded 150 ml of thick, yellow fluid, which was submitted for cytological analyses, a reverse transcription polymerase chain reaction (RT-PCR) for feline infectious peritonitis (FIP), and bacterial culture. The thoracic radiographs after the thoracocentesis illustrated the left pleural effusion (Fig. 1B).

A primer set used for feline coronavirus detection by RT-PCR was 5′-TAA TGC CAT ACA CGA ACC AGC T-3′ and 5′-GTG CTA GAT TTG TCT TCG GAC ACC-3′. Thermocycling conditions consisted of 50 min reverse transcription at 45°C, 5 min incubation at 95°C followed by 33 cycles of 1 min denaturation at 95°C, 1 min primer annealing at 55°C, and 1 min for primer extension at 72°C. The 33 cycles were completed with a 5 min final extension at 72°C. The administration of broad-spectrum antibiotics with amoxicillin clavulanic acid (20 mg/kg [PO] *q* 12 h) was employed pending the antibiotics susceptibility testing. Meloxicam (0.1 mg/kg subcutaneous [SC] *q* 72 h) was prescribed as an anti-inflammatory drug. To correct the dehydration, normal saline NSS [IV] was administered. The cat was followed up 3 days after the first visit, in which the clinical signs persisted. The second thoracocentesis yielded 125 ml of similar thick, yellow fluid.

Results

A CBC revealed decreased hemoglobin and mild normochromic normocytic regenerative anemia with reticulocytosis, leukocytosis, and hyperproteinemia (Table 1). Cytological findings of pleural effusion revealed protein-rich modified transudate with a total nucleated cell count of 1.06×10^3 cells/µl and a specific gravity of 1.042 (Fig. 2). The total protein of the effusion was 6 gm/dl with

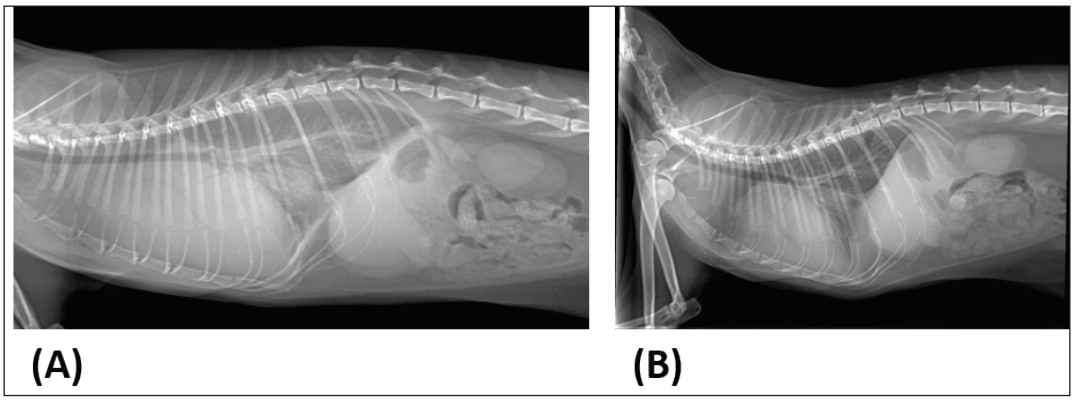

Figure 1. (A) Thoracic radiograph before the thoracocentesis revealed increased dense opacity of the left lung and the presence of a pleural effusion. (B) Thoracic radiograph post-thoracocentesis.

Table 1. Hematology profiles.

Hematology	Result	Reference range
White blood cell	26.07	5.50–19.50 × 10^3 (cells/µl)
Neutrophils	20.86	2.50–12.50 × 10^3 (cells/µl)
Lymphocytes	3.65	1.50–7.0 × 10^3 (cells/µl)
Monocytes	1.56	0.10–1.50 × 10^3 (cells/µl)
Eosinophils	Rare	Rare
Basophils	Rare	Rare
Hemoglobin	8.9	9.80–15.40 (gm/dl)
Hematocrits	28.9	30.00–45.00 (%)
Reticulocyte %	0.96	0.00–0.06 (%)
Protein	9	6.0–8.0 (gm/dl)

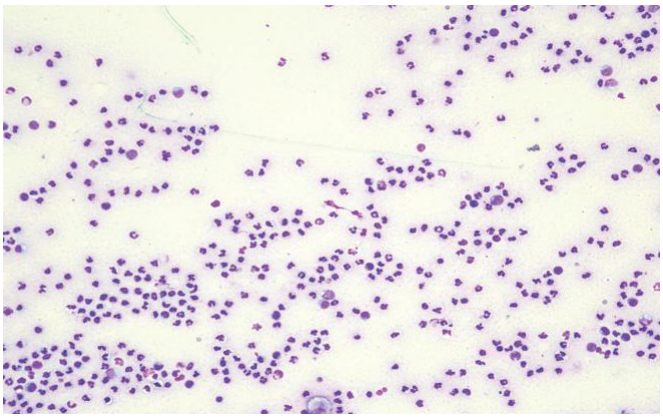

Figure 2. Cytological findings of the thoracic fluid showed non-degenerate neutrophils admixed with foamy macrophages on the prominent eosinophilic stippling granular background.

the albumin and globulin concentration of 1.7 and 3.90 gm/dl, respectively [albumin:globulin (A:G) ratio = 0.4].

The result of RT-PCR testing for FIP of the pleural effusion was negative. The culture of the thoracic fluid revealed a pure growth of smooth, round, convex, and violet-pigmented colonies on both sheep blood agar and MacConkey agar (Fig. 3A and B) after an aerobic incubation at 37°C for 24 h. Gram stain revealed Gram-negative rod-shaped bacteria (Fig. 3C). The biochemical tests were performed, and the biochemical characteristics are shown in Table 2. The triple sugar iron (TSI) test revealed the alkaline slant and acid bottom (K/A) without the production of gas and hydrogen sulfide, which was indicative of dextrose fermentation. The sim motility test showed the diffuse zone of growth expanding from the inoculation line, which indicated the positive motility test. The urease test and indole test indicated that the organism did not express the urease enzyme and tryptophanase enzyme, respectively. The lysine was not decarboxylated. The fermentation test of

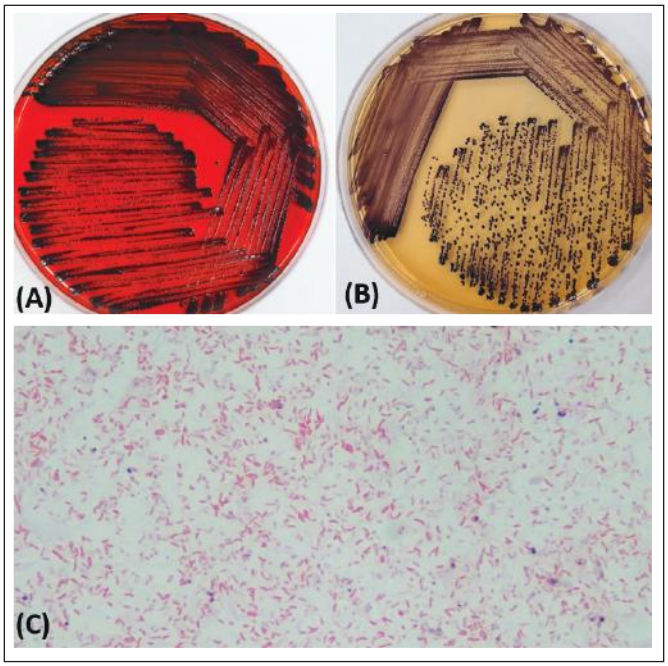

Figure 3. The appearance of *C. violaceum* colonies on sheep blood agar (A) and MacConkey agar (B). (C) Gram stain showed Gram-negative rod-shaped bacteria.

Table 2. Biochemical characteristics of *C. violaceum.*

Biochemical test	Results
TSI	K/A without hydrogen sulfide production
SIM	+
Urea	-
Indole	-
Lysine	-
Arabinose	-
Mannitol	-
Lactose	-
Salicin	-
Raffinose	-
Sucrose	-
Glucose	+

+ = Positive, - = Negative.

arabinose, mannitol, lactose, salicin, raffinose, and sucrose was negative, but the fermentation of glucose was positive. According to the appearance on the medium and biochemical characteristics, the suspected organism was identified as *C. violaceum*. The identification was further confirmed using a matrix-assisted laser desorption ionization-time of flight mass spectrometer (MALDI-TOF MS; VITEK® MS, bioMérieux®). The result of characteristic protein

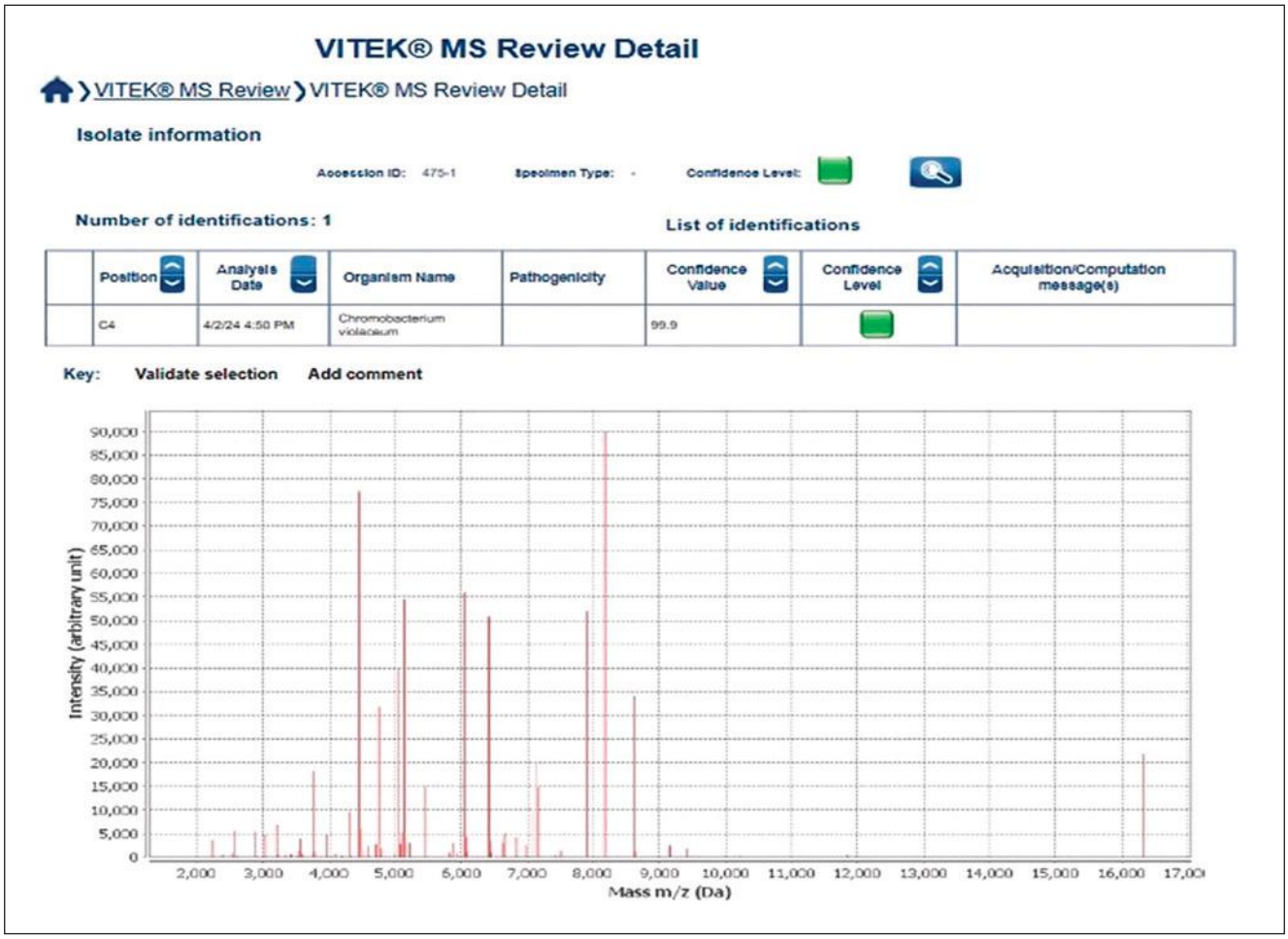

Figure 4. *Chromobacterium violaceum* was identified with a confidence value of 99.9 by MALDI-TOF MS.

fingerprints confirmed *C. violaceum* (Fig. 4). Antimicrobial susceptibility tests were performed using the Kirby-Bauer disc diffusion method, and the results are shown in Table 3. The organism was susceptible to drugs in aminoglycosides, tetracyclines, macrolides, fluoroquinolones, and sulfonamide groups.

Discussion

This case report herein presents *C. violaceum* isolated from the pleural effusion of a cat presented at the veterinary teaching hospital with dyspnea and severe pleural effusion. *Chromobacterium violaceum* is an opportunistic Gram-negative rod-shaped bacterium commonly found in subtropical and tropical environments. In Thailand, a few cases of *C. violaceum* infection have been reported in humans; however, infection in animals has never been addressed before. *Chromobacterium* infection could start with a local infection, which could rapidly disseminate to multiple organs, causing abscesses and septicemia. Previous studies found pleuropneumonia in various animals [13,14,16–18]. In this case, the clinical findings in the cat were similar to previous publications. The characteristics of the effusion revealed protein-rich modified transudate with A:G ratio of 0.4. The culture of the effusion revealed pure isolation of *C. violaceum*, which was presumably responsible for the pleural effusion. Although FIP was considered as a primary differential diagnosis based on cytological findings of the transudate, this report highlights other differential diagnoses, particularly *C. violaceum,* which should be considered as one possibility causing protein-rich modified transudate effusion.

In general, the routes of infection in both humans and animals are either by direct contact or an oral route [14]. Nevertheless, the infection associated with water aspiration during drowning was also reported [22]. In our case, the portal of entry could not be definitively confirmed. The cat lived primarily indoors, but it was able to roam around outside, according to the owner. We speculated that the most likely point of entry could have been direct contact

Table 3. Antimicrobial susceptibility profiles.

Class	Antimicrobial agent	Results
Aminoglycoside	Amikacin	S
	Gentamicin	S
	Kanamycin	S
	Neomycin	S
	Streptomycin	S
Penicillin	Amoxicillin + clavulanic acid	R
	Amoxycillin	R
	Ampicillin	R
	Cloxacillin	R
	Penicillin	R
Cephalosporin	Cephalotin	R
	Cephalexin	R
	Ceftriaxone (third generation)	R
Polymycin	Colistin	R
Lincosamide	Clindamycin	R
	Lincomycin	R
Tetracyclines	Doxycyclin	S
	Oxytetracycline	S
Macrolides	Erythromycin	S
Fluoroquinolones	Enrofloxacin	S
Nitromidazole	Metronidazole	R
Sulfonamide	Sulfamethoxazole + Trimethoprim	S

R = Resistant, S=susceptible.

via broken skin or puncture wounds and contaminated with the environment. Although the external examination found no skin lesions and there was no history of trauma, the wound could have been very small, or the external wound could have been healed and could not be grossly recognized. Moreover, there was a possibility that the cat could have gotten infected while drinking or choking on contaminated water.

Due to a rare incidence of *C. violaceum* infection and no reported pathognomonic lesions, the diagnostic methods and bacteria identification were very challenging. The diagnosis of infection was based on the characteristic of violet pigment production on both sheep blood and MacConkey media, accompanied by biochemical tests (Table 2). To confirm the identification, MALDI-TOF MS posed a promising technology for definitive diagnosis, reducing the turnaround time and the cost for biochemical reagents [23].

Since the infection by *C. violaceum* is frequently associated with fatal outcomes in both humans and animals, early treatment with proper antibiotics was critical. To cover the use of antibiotics in different animal species, 10 different groups of antimicrobials were performed for the susceptibility test. The antimicrobial susceptibility profile was similar to previous publications [17]. The isolate was susceptible to aminoglycosides, tetracyclines, macrolides, fluoroquinolones, and sulfonamides. However, it was alarming that the bacterium commonly found in the environment was resistant to penicillin, cephalosporin, polymyxin, lincosamide, and nitroimidazole. The susceptibility test of *C. violaceum* isolated in this study revealed that cephalosporin did not affect the microorganism, implying that it is a cephalosporin-resistant strain. This study is similar to Nam et al. [24], who mentioned this phenomenon in *C. violaceum* isolated from a human case. Moreover, the isolate revealed resistance to colistin, which is considered a last-resort antibiotic used in humans. This finding was consistent with the findings of Alisjahbana et al. [25], who reported a low proportion (22.2%) of *C. violaceum* susceptible to colistin.

In this case, regarding the gross appearance of the pleural fluid, the broad-spectrum antibiotic amoxicillin + clavulanic acid was chosen to alleviate secondary infections while pending the antimicrobial susceptibility test. Unfortunately, the result of the antimicrobial susceptibility test was delayed because of the rare incidence of *C. violaceum* infection in the cat. As a result, the administration of amoxicillin + clavulanic acid was not discontinued, and the patient was then lost to follow-up.

Conclusion

This report presented the first *C. violaceum* infection in animals in Thailand. It is considered an opportunistic bacterium in both veterinary and human medicine. The isolation was susceptible to drugs in aminoglycosides, tetracyclines, macrolides, fluoroquinolones, and sulfonamide groups. The presence of this case is a significant public health concern, particularly as these animals can act as reservoirs for pathogens with the potential to infect immunocompromised individuals, which can cause the transmission of zoonotic pathogens.

List of abbreviations

A:G, albumin:globulin; °C, degree Celsius; CBC, Complete blood count; dl, deciliter; FIP, Feline infectious peritonitis; gm, grams; h, hour; kg, kilogram; MALDI-TOF MS, matrix assisted laser desorption ionization-time of flight mass spectrometer; mg, milligram; min, minutes; ml, milliliter; RT-PCR, Reverse transcription polymerase chain reaction; sec, seconds; µl, microliter.

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Veterinary Medicine, for their hard work and for dedicating the time to provide quality testing and good services in this case.

Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contributions

ND was involved in the physical examination and sample collection. SK, SC, and MS were involved in microbiological testing. PE was involved in the interpretation of the pathological finding. AA took part in the interpretation of microbiological findings and in preparing this manuscript.

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