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【 CASE REPORT 】

Putative Novel Species of Genus *Capnocytophaga*, *Capnocytophaga stomatis* Bacteremia in a Patient with Multiple Myeloma after Direct Contact with a Cat

Koh Shinohara[1,2], Yasuhiro Tsuchido[2,3], Michio Suzuki[4], Kojiro Yamamoto[5], Yasutaro Okuzawa[6], Koichi Imaoka[4] and Tsunehiro Shimizu[1]

Abstract:

Capnocytophaga species are among the typical zoonotic pathogens causing infections following direct contact with animals. Recently, a putative novel species of zoonotic *Capnocytophaga*, *Capnocytophaga stomatis*, was reported. We herein report the first case of bacteremia caused by *C. stomatis*. A woman in her 80s with multiple myeloma who was receiving bortezomib and dexamethasone therapy was admitted to our hospital with a 2-day history of a fever and right calf redness. She was often licked by her cat. On a blood culture, thin, Gram-negative rods were detected, which were identified as *C. stomatis* by whole-genome sequencing. The patient was successfully treated with ampicillin-sulbactam treatment. Our case highlights the pathogenic potential of the putative novel *Capnocytophaga*, *C. stomatis*, in immunocompromised hosts.

Key words: *Capnocytophaga stomatis*, cellulitis, multiple myeloma, bacteremia, whole genome sequencing

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Introduction

Capnocytophaga species are fermentative bacteria that appear as slender, Gram-negative bacilli and are divided into two distinct groups. *Capnocytophaga ochracea, C. sputigena, C. haemolytica, C. granulosa, C. leadbetteri, C. gingivalis,* and *C. periodontitidis* are among the groups associated with the human oral cavity, while *C. canimorsus, C. canis, C. cynodegmi,* and *C. felis* are zoonotic (1-3). Recently, Zangenah et al. reported a putative novel species of the genus *Capnocytophaga*, *Capnocytophaga stomatis*, using whole-genome sequencing (4). *C. stomatis* has been isolated in humans who developed wound infections following animal bites and is genetically similar to *C. cynodegmi*. However, clinical information concerning *C. stomatis* is lacking, and its pathogenic potential in humans is unclear.

We herein report the first case of *C. stomatis* bacteremia, identified using whole-genome sequencing.

Case Report

A woman in her 80s presented to our hospital with a 2-day fever history and right calf redness. Two years earlier, she had been diagnosed with asymptomatic multiple myeloma. The patient had experienced worsening bilateral leg edema over the past six months. Furthermore, a urinalysis revealed proteinuria, consistent with nephrotic syndrome. A renal biopsy confirmed amyloid light-chain amyloidosis. Bortezomib and dexamethasone were administered four weeks before the presentation. The patient kept a cat in her house, which often licked and scratched her calves. In addition to bilateral leg edema, redness from the right ankle to the distal half of the calf was observed. Vital signs showed a low-grade fever (37.1°C) and mild tachycardia (96 beats per minute).

[1]Department of Infectious Diseases, Kyoto City Hospital, Japan, [2]Department of Clinical Laboratory Medicine, Kyoto University Graduate School of Medicine, Japan, [3]Department of Infectious Diseases, University Hospital, Kyoto Prefectural University of Medicine, Japan, [4]Department of Veterinary Science, National Institute of Infectious Diseases, Japan, [5]Department of Nephrology, Kyoto City Hospital, Japan and [6]Department of Dermatology, Kyoto City Hospital, Japan
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Correspondence to Dr. Koh Shinohara, shinoharakoh@kuhp.kyoto-u.ac.jp

Table 1. Results of the Laboratory Examination on Admission.

Variables		Reference range, adult	Variables		Reference range, adult
White blood cell count (/μL)	6,570	3,500-8,500	Alkaline phosphatase (U/L)	218	110-350
Red blood cell count (×10⁴/μL)	339	380-490	Lactate dehydrogenase (U/L)	391	120-230
Hemoglobin (g/dL)	11.8	11.5-15.0	Creatinine (mg/dL)	1.97	0.3-1.1
Hematocrit (%)	34.1	34-45	Blood urine nitrogen (mg/dL)	48.5	8-21
Platelet count (×10³/μL)	800	1,300-3,500	Natrium (mEq/L)	132	135-147
Total protein (g/dL)	4.8	6.7-8.3	Potassium (mEq/L)	5.3	3.3-4.8
Albumin (g/dL)	1.8	3.9-4.9	Chloride (mEq/L)	104	98-109
Total bilirubin (mg/dL)	0.6	0.2-1.2	Calcium (mg/dL)	8.1	8.2-10.2
Aspartate aminotransferase (U/L)	31	0-35	C-reactive protein (mg/dL)	0.09	0-0.3
Alanine aminotransferase (U/L)	18	0-30			

A laboratory examination on admission revealed unremarkable findings concerning the white blood cell count (6,570/μL) and serum C-reactive protein level (0.09 mg/dL). The results of laboratory tests are summarized in Table 1. Cellulitis was diagnosed, and cefazolin (1 g every 12 h) was administered. Although defervescence was observed on admission day 2, the local signs of cellulitis were not resolved. On admission day 4, Gram-negative rod-shaped bacteria were isolated from two sets of anaerobic blood culture obtained on admission. Cefazolin was replaced with intravenous ampicillin-sulbactam (3 g every 12 h) on admission day 5, after which the local signs of cellulitis improved. Follow-up blood cultures, which were obtained at admission days 10 and 15, were negative, and transthoracic echocardiography showed no evidence of infective endocarditis. The seven-day ampicillin-sulbactam treatment was followed by oral amoxicillin-clavulanic acid, and the patient was discharged on admission day 13.

However, she was readmitted on day 14 because of skin eruption and a fever caused by amoxicillin-clavulanic acid, and ceftriaxone was administered instead. The 14-day therapy after the initiation of ampicillin-sulbactam was completed, and no relapse was observed thereafter. The clinical course is shown in Fig. 1.

Microbiology

The isolates recovered from blood cultures were identified as *C. cynodegmi* by matrix-associated laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS; Microflex LT Biotyper; Bruker Daltonics, Bremen, Germany). The identification score was 1.70. The isolate was further examined by a 16S rRNA gene sequence analysis. The 1,383-bp sequencing product was compared with sequences deposited in the EzTaxon database, which resulted in a 99.3% identity match with the *C. cynodegmi* strain (DSM 19736). The isolate was confirmed to be *C. cynodegmi* using species-specific polymerase chain reaction (PCR) methods (5). A whole-genome sequencing method was applied for further investigation. The average nucleotide identity (ANI), measured using the JSpecies Web Server (JSpeciesES, https://www.ribocon.com/jspeciesws.html) (6). The ANI represents the average nucleotide identity of all orthologous genes shared between any two genomes and offers robust resolution between strains of the same or closely related species. In general, an ANI value 95% is equivalent to DDH value 70%, which is regarded as the threshold for differentiation of the species. The ANI value between our isolate (HP26001) and the *C. stomatis* strain H2177 was 97.2%, whereas the values between our isolate and DSM 107251 (a type strain of *C. felis*) and between our isolate and ATCC 49044 (a type strain of *C. cynodegmi*) were 89.2% and 84.1%, respectively. A whole-genome-based phylogenetic analysis of the isolated genome was performed with the Type (Strain) Genome Server (https://tygs.dsmz.de) to confirm close phylogenetic relationships between our isolate and the H2177 strain (Fig. 2). These results suggested that our isolate and H2177 were genetically different from *C. cynodegmi*. The isolate did not exhibit hemolytic activity on sheep blood agar plates (Fig. 3). The isolates were also re-analyzed using MALDI-TOF MS 6 times, which generated a score between 1.47-1.68 for *C. cynodegmi*. An E-test strip (bioMérieux, Marcy-L'Etoile, France) and the disk diffusion test were used to determined antimicrobial susceptibility following incubation on sheep blood agar [Try/Soy Blood Agar (Sheep) No.2, Kyokuto Pharmaceutical Industrial, Tokyo, Japan] at 37°C in 5% CO_2 for 48 h (Table 2) (7). Although there is no standard *Capnocytophaga* species susceptibility testing method, it appears resistant to gentamicin. When applying the Clinical and Laboratory Standards Institute (CLSI) clinical breakpoints for Enterobacterales (8), the zone diameters of beta lactams were regarded as susceptible; however, that of cefazolin (18 mm) was included in the resistant category (≤19 mm).

Discussion

C. stomatis is a putative novel species of zoonotic *Capnocytophaga*, described by Zangenah et al. (4). It was found to be pathogenic in humans when isolated from an infected wound caused by a dog bite. However, no severe disease cases, including bacteremia caused by *C. stomatis*, have been reported. Our case revealed the pathogenic potential of

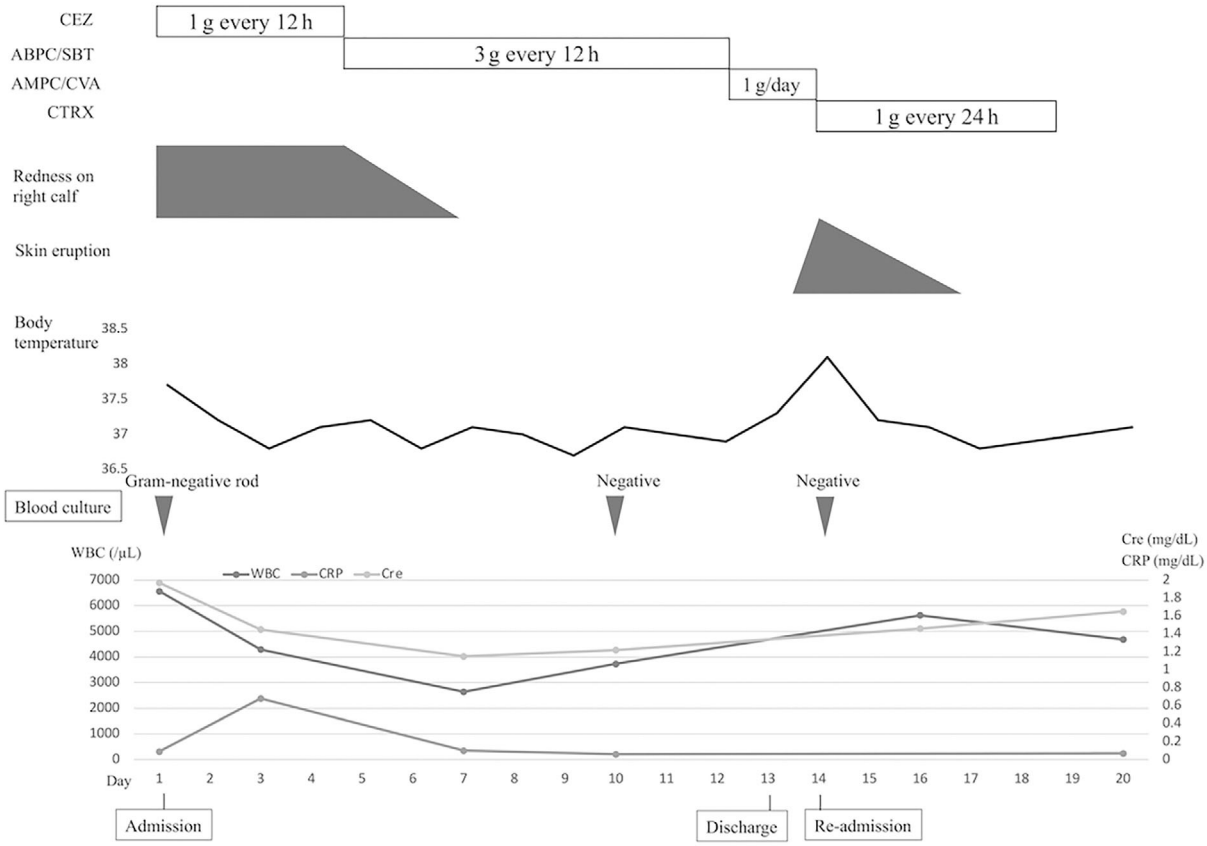

Figure 1. **The clinical course of this case. CEZ: cefazolin, ABPC/SBT: ampicillin/sulbactam, AMPC/CVA: amoxicillin/clavuate acid, CTRX: ceftoriaxone, WBC: white blood cell count, CRP: C-reactive protein, Cre: serum creatinine**

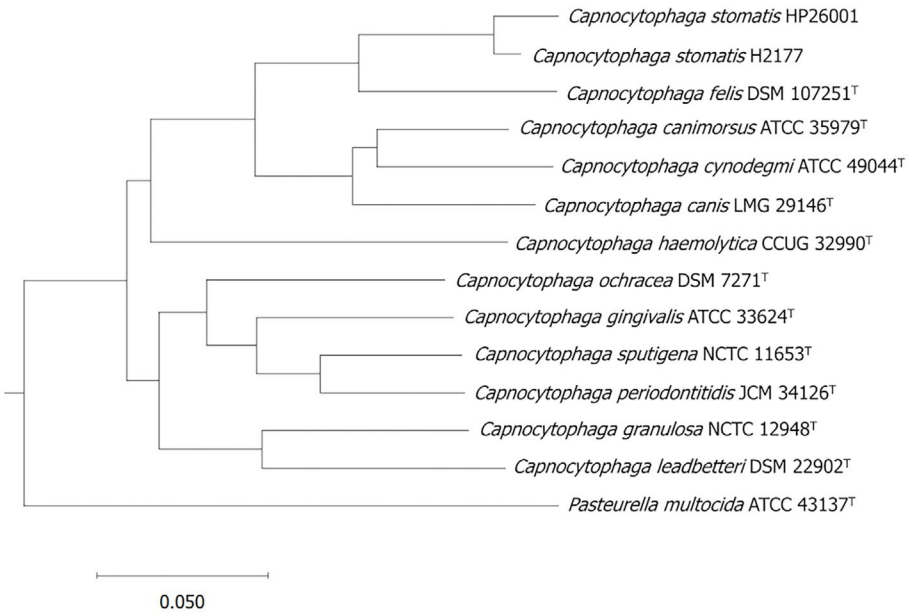

Figure 2. **Tree inferred with FastME 2.1.6.1 from GBDP distances calculated from whole-genome sequences of the "*Capnocytophaga stomatis*" strain HP26001 and related species. The tree was rooted at the midpoint using *Pasteurella multocida* as an outgroup sequence. The branch lengths are scaled in terms of the GBDP distance formula d5.**

C. stomatis to cause severe infection in immunocompromised hosts.

Most human infections with *Capnocytophaga* species are caused by *C. canimorsus*, and documented cases of bactere-

mia of non-*carnimorsus Capnocytophaga* are limited. To our knowledge, only two cases of *C. cynodegmi* (9, 10) and four cases of *C. canis* (7, 11-14) have been reported (Table 3). Most cases of non-*canimorsus Capnocytophaga* bacteremia occurred in immunocompromised patients, especially anatomical asplenic patients (7, 10, 13, 14) or those with a splenic function reduction due to liver diseases (7, 11-13). In our case, humoral immune deficiency due to multiple myeloma and nephrotic syndrome, combined with cellular immune deficiency due to steroid and bortezomib use, appeared to have caused bacteremia. In addition, all non-*canimorsus Capnocytophaga* bacteremia cases, including our case, were associated with direct contact with cats or dogs, not limited to bites (7, 9-14). Physicians should carefully identify patient's animal exposure history when managing cases of skin and soft tissue infections.

Our isolate's MALDI-TOF MS log score was below the cut-off level of 1.7. Zangenah et al. reported the usefulness of MALDI-TOF analysis for *C. canimorsus* or *C. cynodegmi* identification (15); however, among the strains identified as *C. cynodegmi*, three *C. stomatis* and one *C. canis* strains were included, which generated log scores below 1.7 (4, 15). Our isolate also exhibited log scores of 1.47-1.70. Based on these results, a MALDI-TOF analysis may contribute to identifying *C. stomatis* and *C. cynodegmi*. Zangenah et al. described the colony morphologies of *C. stomatis* on blood agar plates. The colonies of *C. stomatis* were reportedly flat, larger than those of *C. cynodegmi*, and formed transparent/greyish colonies similar to those of *C. canimorsus*, which were clearly distinct from those of other zoonotic *Capnocytophaga* sp. In addition, the isolates of *C. stomatis* exhibited beta hemolytic activity on blood agar. However, our isolate showed gliding motility and no beta hemolytic activity, suggesting that the colony morphologies and hemolytic activity of *C. stomatis* isolates are not uniform, and attention should be paid to the identification of *Capnocytophaga* species by phenotypic features.

Suitable antimicrobial agents for *C. stomatis* have not yet been determined. Historically, zoonotic *Capnocytophaga* species, mainly *C. canimorsus*, have been broadly susceptible to most antimicrobial agents, and penicillin or penicillin/beta-lactamase inhibitor combination is recommended as first-line therapy. However, recent reports have revealed that

Figure 3. Colony morphology of the isolate on sheep blood agar, 48 h culture, 37 °C, 5% CO_2 atmosphere.

Table 2. The Results of Antibiotic Susceptibility Test of the Isolate.

Antimicrobial agents	Disk (mm)	E-test
Penicillin G	27	0.125
Amoxicillin/clavuate acid	25	0.094
Cefazolin	18	-
Ceftriaxone	34	0.032
Imipenem	36	0.25
Gentamycin	-	>256
Minocycline	39	0.047
Ciprofloxacin	23	0.75
Azithromycin	28	-
Clindamycin	36	-

Table 3. Literature Review of the Cases with Non-*canimorsus* Zoonotic *Capnocytophaga* Species Bacteremia.

Case No. [Ref.]	Organism	Age	Sex	Underlying illness	Animal exposure	Source of isolation	Clinical manifestation	Outcome
1 [9]	*C. cynodegmi*	59	Male	Diabetes mellitus	Dog bite	Blood, BALF, sputum, pus	Cellulitis, sepsis, pneumonitis	Recovered
2 [10]	*C. cynodegmi*	72	Female	Post-splenectomy	Dog bite	Blood, CSF	Septic shock, meningitis	Died
3 [11, 14]	*C. canis*	49	Male	Chronic alcoholic consumption	Cat scratch	Blood	Sepsis	Recovered
4 [7, 14]	*C. canis*	67	Female	Idiopathic portal hypertension, post-splenectomy	Cat bite	Blood	Septic shock	Recovered
5 [12, 14]	*C. canis*	82	Female	Liver cancer	Contact with dog	Blood	Sepsis, multiple organ failure	Died
6 [13]	*C. canis*	70	Male	Atrial fibrillation, chronic alcoholic consumption, post-splenectomy	Cat scratch	Blood	Septic shock	Recovered
This case	*C. stomatis*	81	Female	Multiple myeloma, nephrotic syndrome	Contact with cat	Blood	Cellulitis	Recovered

CSF: cerebrospinal fluid, BALF: bronchoalveolar lavage fluid

some strains of *Capnocytophaga* species, mainly human *Capnocytophaga* species and zoonotic *Capnocytophaga* species, harbor beta-lactamases, such as OXA-347, and demonstrate resistance to penicillin, cephalosporins, and imipenem (16). Although our isolate appeared to be susceptible to other beta-lactams, the zone diameter of cefazolin (18 mm) is regarded as resistant when using the CLSI clinical breakpoints for Enterobacterales (8), and the clinical response to cefazolin in this case appeared partially ineffective. More clinical information and antimicrobial susceptibility data of zoonotic *Capnocytphaga* species are needed.

Our case highlights the pathogenic potential of a putative novel *Capnocytophaga*, *C. stomatis*, in immunocompromised hosts. Since 16S rRNA sequencing and species-specific PCR cannot differentiate between *C. cynodegmi* and *C. stomatis*, molecular surveillance using whole-genome sequencing will help deepen our understanding of the clinical and epidemiological features of zoonotic *Capnocytophaga* infections.

The authors state that they have no Conflict of Interest (COI).

References

1. Brenner DJ, Hollis DG, Fanning GR, Weaver RE. *Capnocytophaga canimorsus* sp. nov. (formerly CDC group DF-2), a cause of septicemia following dog bite, and *C. cynodegmi* sp. nov., a cause of localized wound infection following dog bite. J Clin Microbiol **27**: 231-235, 1989.
2. Suzuki M, Umeda K, Kimura M, Imaoka K, Morikawa S, Maeda K. *Capnocytophaga felis* sp. nov. isolated from the feline oral cavity. Int J Syst Evol Microbiol **70**: 3335-3360, 2020.
3. Zhang Y, Qiao D, Shi W, Wu D, Cai M. *Capnocytophaga periodontitidis* sp. nov., isolated from subgingival plaque of periodontitis patient. Int J Syst Evol Microbiol **71**: 2021.
4. Zangenah S, Abbasi N, Andersson AF, Bergman P. Whole genome sequencing identifies a novel species of the genus *Capnocytophaga* isolated from dog and cat bite wounds in humans. Sci Rep **6**: 22919, 2016.
5. Suzuki M, Kimura M, Imaoka K, Yamada A. Prevalence of *Capnocytophaga canimorsus* and *Capnocytophaga cynodegmi* in dogs and cats determined by using a newly established species-specific PCR. Vet Microbiol **144**: 172-176, 2010.
6. Ritchter M, Rosselló-Móra R, Glöckner FO, Peplies J. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. Bioinformatics **32**: 929-931, 2015.
7. Taki M, Shimojima Y, Nogami A, et al. Sepsis caused by newly identified *Capnocytophaga canis* following cat bites: C. canis is the third candidate along with *C. canimorsus* and *C. cynodegmi* causing zoonotic infection. Intern Med **57**: 273-277, 2018.
8. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 31st ed. Clinical and Laboratory Standards Institute, Wayne, PA, 2021.
9. Sarma PS, Mohanty S. *Capnocytophaga cynodegmi* cellulitis, bacteremia, and pneumonitis in a diabetic man. J Clin Microbiol **39**: 2028-2029, 2001.
10. Khawari AA, Myers JW, Ferguson DA Jr, Moorman JP. Sepsis and meningitis due to *Capnocytophaga cynodegmi* after splenectomy. Clin Infect Dis **40**: 1709-1710, 2005.
11. Yamamoto U, Kunita M, Mohri M. Shock following a cat scratch. BMJ Case Rep **2013**: bcr2012007892, 2013.
12. Irie Y, Yamatogi T, Sugahara R, Suzuki M, Imaoka K. A case of septicemia caused by putative novel species of genus Capnocytophaga. Nihon Rinsho Biseibutsugaku Zasshi (J Jpn Soc Clin Microbiol) **25**: S1 290, 2015 (in Japanese).
13. Donner V, Buzzi M, Lazarevic V, et al. Septic shock caused by *Capnocytophaga canis* after a cat scratch. Eur J Clin Microbiol Infect Dis **39**: 1993-1995, 2020.
14. Suzuki M, Imaoka K, Haga Y, et al. Characterization of three strains of *Capnocytophaga canis* isolated from patients with sepsis. Microbiol Immunol **62**: 567-573, 2018.
15. Zangenah S, Özenci V, Boräng S, Bergman P. Identification of blood and wound isolates of *C. canimorsus* and *C. cynodegmi* using VITEK2 and MALDI-TOF. Eur J Clin Microbiol **31**: 2631-2637, 2012.
16. Zangenah S, Andersson AF, Özenci V, Bergman P. Genomic analysis reveals the presence of a class D beta-lactamase with broad substrate specificity in animal bite associated *Capnocytophaga* species. Eur J Clin Microbiol Dis **36**: 657-662, 2017.