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Bloodstream Infection Caused by *Actinomyces neuii* subsp. *anitratus* in a Patient with Breast Cancer: A Case Report and Literature Review

Toshinori HARA[1,2,3,*)], Hiroki KITAGAWA[3,4,5)], Toshiki KAJIHARA[3,6)], Yumiko KOBA[1,2,3)], Kayoko TADERA[1,2,3)], Rie NAGAOKA[1,2)], Shiho OTA[1,2)], Yusuke NAKAOKA[1,2)], Toshihito NOMURA[4)], Keitaro OMORI[4)], Norifumi SHIGEMOTO[3,4,5,7)], Shinsuke SASADA[8)], Kohei OTA[9)], Seiya KASHIYAMA[1,2,3)], Michiya YOKOZAKI[2,3)], Nobuaki SHIME[9)], and Hiroki OHGE[3,4)]

1) Section of Infection Diseases Laboratory, Department of Clinical Support, Hiroshima University Hospital, Hiroshima, Japan
2) Division of Clinical Laboratory Medicine, Hiroshima University Hospital, Hiroshima, Japan
3) Project Research Center for Nosocomial Infectious Diseases, Hiroshima University, Hiroshima, Japan
4) Department of Infectious Diseases, Hiroshima University Hospital, Hiroshima, Japan
5) Department of Surgery, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan
6) Department of Antimicrobial Resistance Research Center, National Institute of Infectious Diseases, Tokyo, Japan
7) Translational Research Center, Hiroshima University, Hiroshima, Japan
8) Department of Surgical Oncology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan
9) Department of Emergency and Critical Care Medicine, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan

ABSTRACT

Actinomyces neuii can grow under aerobic culture conditions and shows a gram-positive rod morphology, similar to that of *Corynebacterium* spp. *A. neuii* is usually detected in local pus samples, and published cases of *A. neuii* bloodstream infections are rare. Here, we report a case of bloodstream infection caused by *A. neuii* subsp. *anitratus*. A 53-year-old woman with fever and hypotension was referred to our hospital. The patient underwent surgery for breast cancer and received chemotherapy after central venous (CV) port placement. On day 2, a blood culture in an anaerobic bottle yielded positive results, and *A. neuii* subsp. *anitratus* was identified via matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI–TOF MS) and 16S rRNA sequencing. The patient was diagnosed with bloodstream infection caused by *A. neuii* subsp. *anitratus* with CV port infection. The CV port was removed and antibiotic treatment resulted in symptom improvement so the patient was discharged on day 28 of hospitalization. MALDI–TOF MS and 16S rRNA sequencing were found to be more useful for the identification of *A. neuii* than for phenotypic identification. Further research on *A. neuii* subsp. *anitratus* infections is required to avoid delayed or missed diagnoses.

Key words: *A. neuii subsp. anitratus*, *Gram-positive rods*, *MALDI–TOF MS*, *16S rRNA sequencing*

INTRODUCTION

Actinomyces spp are gram-positive anaerobic rods and are known to be the causative pathogens of actinomycosis. *Actinomyces* is a common inhabitant of the oral cavity, gut, genitourinary tract, and skin[9)]. Among the *Actinomyces* spp, *A. israelii* and *A. gerencseriae*, which are isolated at comparatively high rates, require anaerobic culturing, and characteristically appear as spider-like structures on Gram staining. In contrast, *A. neuii* grows under aerobic culture conditions and presents a coryneform gram-positive rod morphology on Gram staining[18)]. *A. neuii and Corynebacterium* spp share common microbiological features such as growth conditions, Gram staining pattern, and slow growth, and so it is difficult to distinguish between them. *A. neuii* causes various types of infections, including breast abscess[11)], urogenital tract infection[4)], endocarditis[3,7)], and endophthalmitis[15)]. In addition, *A. neuii* is usually detected in local pus samples, and published cases of *A. neuii* bloodstream infections are rare[19)]. In this paper, we report a case of bloodstream infection caused by *A. neuii* subsp. *anitratus* through an infected central venous (CV) port.

* Corresponding author: Toshinori Hara
Hiroshima University Hospital, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan
Tel: +81-82-257-5546, Fax: +81-82-257-1579, E-mail: t2128@hiroshima-u.ac.jp

CASE REPORT

A 53-year-old woman with fever and hypotension was referred to our hospital. She had a medical history of scleroderma and interstitial pneumonia, which did not require steroid medication. The patient had undergone right mastectomy for right breast cancer (T4bN0M0, stage IIIB) and axillary lymphadenectomy 2 months prior. Adjuvant chemotherapy, docetaxel, and cyclophosphamide were administered 2 weeks after the CV port placement. She was examined by a local physician due to physical deconditioning one week prior. Levofloxacin was prescribed, but the patient did not take the medication. One day prior, after visiting a local physician, she developed a fever (38.9°C) and hypotension. She was referred to our hospital for further treatment. Upon arrival at the hospital, the patient presented with a blood pressure of 66/40 mmHg, body temperature of 37.2°C, respiratory rate of 30 cycles/min, peripheral arterial oxygen saturation of 97% (while receiving 3 L/min of oxygen via a face mask), and heart rate of 130 beats/min. Laboratory testing revealed a white blood cell count of 84,030/μL with a neutrophil percentage of 96% and a C-reactive protein level of 17.62 mg/dL. The quick Sequential Organ Failure Assessment (qSOFA) and SOFA scores on admission were 2 and 9, respectively. Physical examination revealed redness and pain in the skin at the site of CV port insertion into the left subclavian vein (Figure 1).

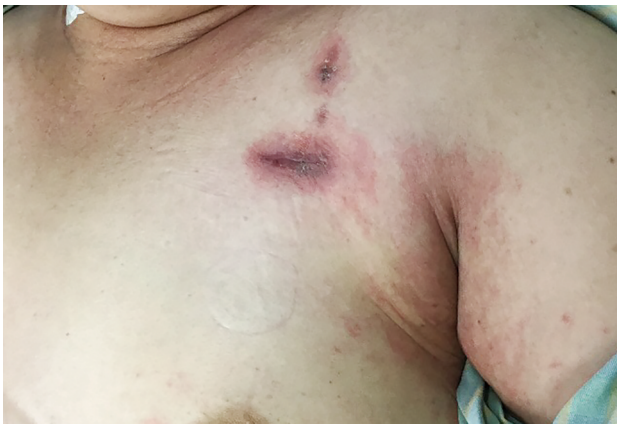

Figure 1 Reddening of the skin at the site of central venous port insertion into the left subclavian vein.

Plain computed tomography revealed no remarkable changes in the chest or abdominal areas. Therefore, CV port infection was suspected. The patient required large amounts of intravenous fluids and norepinephrine to manage the hypotension and was admitted to the intensive care unit. After admission, the CV port was removed, and no abscess was found at the surgical site. Cultures of the catheter tip, urine, and two blood samples were collected. Gram staining revealed no bacteria in the cultured samples of the catheter tip or urine. Empirical antimicrobial therapy consisting of teicoplanin (TEIC) (400 mg every 12 h, intravenously) and cefepime (CFPM) (2 g every 24 h, intravenously) was initiated. On day 2, a blood culture of the anaerobic bottle (BacT/ALERT 3D, bioMerieux, Marcy-l'Étoile, France) yielded positive results, and Gram staining of the sample showed club-form palisade V-shaped gram-positive short coryneform rods (Figure 2), and so infection with *Corynebac≠*

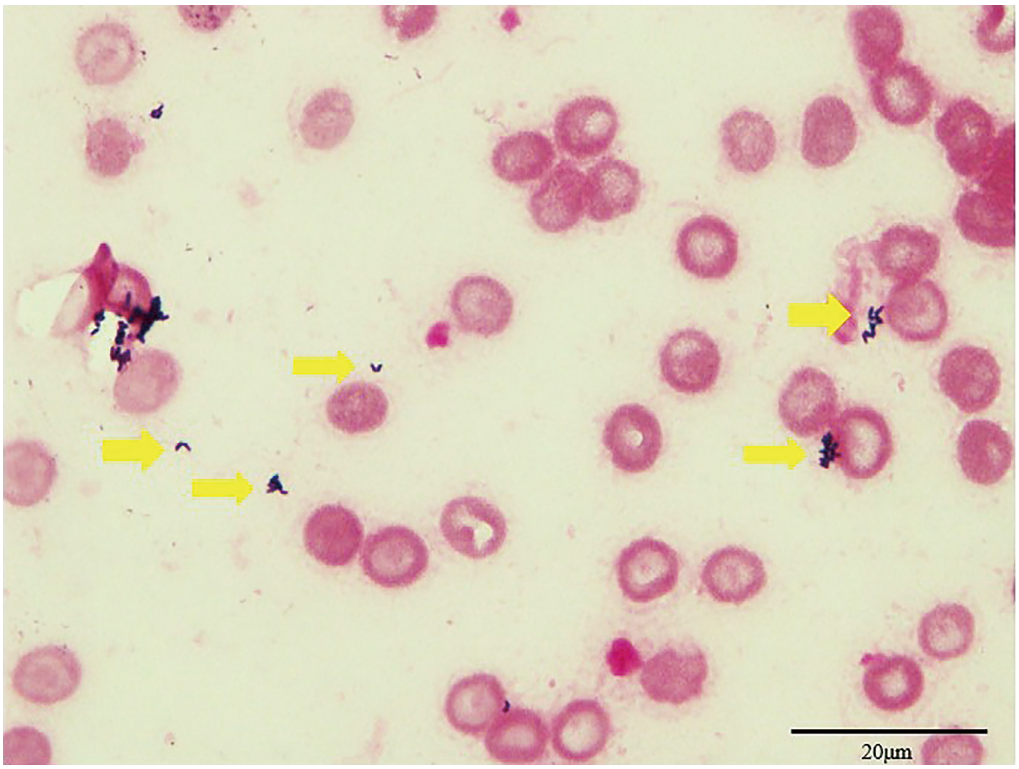

Figure 2 Blood culture sample of *Actinomyces neuii* subsp. *anitratus* by gram staining. Gram stain: Bartholomew and Mittwer method ×1000. Yellow arrow: gram-positive rods (coryneform rods).

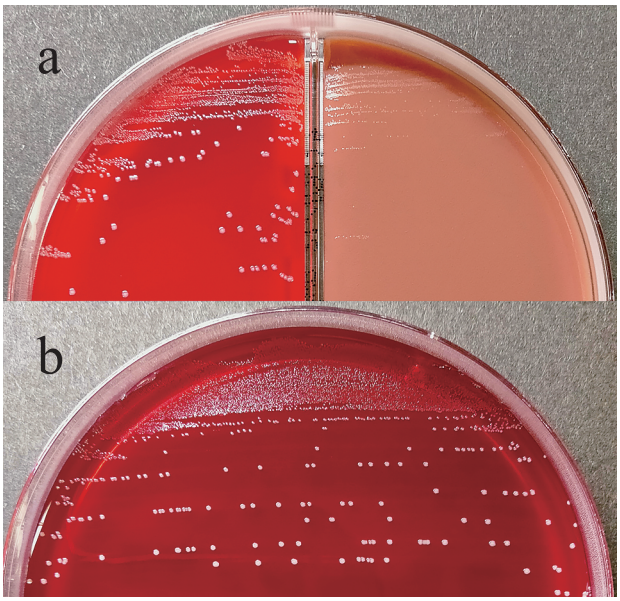

Figure 3 Colonies of *Actinomyces neuii* subsp. *anitratus* incubated on agar plates. a: 5% sheep blood and chocolate agar. b: Brucella HK agar.

terium spp was suspected.

A culture of the positive blood culture sample was performed on 5% sheep blood and chocolate agar (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan) at 35°C in 5% CO_2 and Brucella HK agar (Kyokuto Pharmaceutical Industries Industrial Co., Ltd.) at 35°C under anaerobic conditions. After 48 h of culture, minute pale white colonies were observed on 5% sheep blood, chocolate agar, and Brucella HK agar (Figure 3).

Extended incubation was performed for 5 days, but the colonies were small on the chocolate agar medium. In contrast, the anaerobic culture yielded better strain growth than the aerobic culture. In addition, cultures of the catheter tip and urine yielded negative results. The causative pathogen was identified using a Vitek2 Compact Automated System (bioMerieux) with anaerobe and *Corynebacterium* identification cards and matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI–TOF MS) using the MALDI Biotyper system (Bruker Daltonik GmbH, Bremen, Germany). The Vitek2 Compact system identified *A. neuii* (identification rate: 98%) and MALDI-TOF MS identified *A. neuii* subsp. *anitratus* (score value: 2.436). Basic Local Alignment Search Tool (BLAST) analysis based on the 16S rRNA sequence identified *A. neuii* subsp. *anitratus* (homology of 99% to *Actinomyces neuii* subsp. *anitratus* strain DSM8577, GenBank accession number: NR042429). In addition, we attempted identification using API Coryne (bioMérieux). The first candidates were *Cellulomonas* spp and *Microbacterium* spp (identification rate: 85.5%), and the second candidate was *A. neuii* subsp. *neuii* (identification rate: 14.4%), showing a lack of identification precision.

The minimum inhibitory concentration (MIC) values of various antimicrobial agents were determined using the E-test (bioMerieux) (Table 1).

Antimicrobial susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines[2,13]. However, these MIC values were used for reference only because the interpretation of MICs determined using the E-test is not mentioned in the CLSI M24-A criteria[13]. Although the culture of the catheter tip returned negative results, based on the patient's condition, she was diagnosed with a bloodstream infection caused by *A. neuii* subsp. *anitratus* due to CV port infection.

Table 1 Results of antimicrobial susceptibility testing

Antimicrobial agents	Range	MIC (μg/mL)
Ampicillin	0.016–256	0.016
Penicillin	0.002–32	0.012
Ceftriaxone	0.016–256	0.0.23
Imipenem	0.002–32	0.023
Meropenem	0.002–32	0.004
Clindamycin	0.016–256	> 256
Azithromycin	0.016–256	> 256
Clarithromycin	0.016–256	3
Vancomycin	0.016–256	0.38
Teicoplanin	0.016–256	0.016
Linezolid	0.016–256	0.25
Metronidazole	0.016–256	> 256

MIC, minimum inhibitory concentration

The patient was treated with TEIC and CFPM for 10 days. Antibiotic susceptibility test results were obtained on day 6, owing to slow growth. Once the susceptibility test results were available, upon consultation with the attending physician, empiric treatment was continued without de-escalation because the patient's physical condition improved. Repeated blood culture results obtained on day 20 were negative. The patient improved clinically and was discharged on day 28 of hospitalization.

DISCUSSION

We encountered a case of bloodstream infection caused by *A. neuii* subsp. *anitratus* due to CV port infection in a patient with breast cancer. *A. neuii* a member of the *Actinomyces* spp, appear as Gram-positive rods, whose identification in clinical microbiology laboratories can be challenging due to its similarity to *Corynebacterium* spp in terms of Gram stain images, growth conditions under aerobic culture and slow growth. *A. neuii* was classified as *A. neuii* subsp. *neuii* and *A. neuii* subsp. *anitratus*. Funke G, et al. defined *A. neuii* and *A. anitratus* by studying their metabolic and cellular fatty acid patterns and 16S rRNA sequencing in 1994[5]. In addition, *A. neuii* and *A. anitratus* can be differentiated by their nitrate-reducing abilities[16]. However, because the Vitek2 Compact system cannot analyze the nitrate-reducing ability, it is not possible to differentiate between *A. neuii* and *A. anitratus* using this method. In this case, the Vitek2 Compact system accurately identified *the species* level; however, it could not distinguish between *A. neuii and A. anitratus*. While API Coryne can usually identify *the A. neuii* subspecies level, in this case, identification was not possible because

Table 2 Clinical characteristics of patients with *Actinomyces neuii* bloodstream infection

Case No. [reference]	Age (years)	Sex	Comorbidities	Primary infection site	Organism	Antibiotics	Treatment period	Outcome
1 [6]	62	Female	Chronic schizophrenia	Septic arthritis or urinary tract infection	*Actinomyces neuii*	Ciprofloxacin, imipenem	28 days	Died due to pulmonary embolism
2 [12]	0	Female	Prematurely delivered	Chorioamnionitis	*Actinomyces neuii* subsp. *neuii*	Ampicillin, gentamicin, penicillin	6 weeks	Cured
3 [3]	68	Male	None	Infective endocarditis	*Actinomyces neuii*	Ampicillin, gentamicin, ceftriaxone	Not described	Cured
4 [8]	91	Male	Chronic nephropathy	Cystitis	*Actinomyces neuii* subsp. *neuii*	Cefuroxime, mecillinam	9 days	Cured
5 [8]	67	Male	Surgery for ureteric stenosis	Perirenal abscess	*Actinomyces neuii* subsp. *neuii*	Ampicillin, penicillin, ciprofloxacin	37 days	Cured
6 [7]	66	Male	Aortic valve insufficiency, thoracic aortic aneurysm, diabetes mellitus	Prosthetic valve endocarditis	*Actinomyces neuii* subsp. *neuii*	Penicillin G, meropenem, erythromycin, amoxicillin	365 days	Cured
7 [4]	26	Male	Imperforated anus and bladder neck fistula repair	Postoperative testicular abscess	*Actinomyces neuii*	Vancomycin, gentamicin, clindamycin, piperacillin/tazobactam, ertapenem	Not described	Cured
8 [14]	57	Female	Cervical cancer	Vertebral osteomyelitis	*Actinomyces neuii*	Penicillin	6 weeks	Cured
9 [20]	61	Male	End-stage renal disease, monoclonal gammopathy	Infective endocarditis	*Actinomyces neuii*	Vancomycin, piperacillin/tazobactam, ampicillin, gentamicin, doxycycline	23 months	Cured
10 [1]	0	Female	Prematurely delivered	Unknown	*Actinomyces neuii*	Ampicillin, gentamycin, penicillin	6 weeks	Cured
Present case	53	Female	Breast cancer	Central venous port infection	*Actinomyces neuii* subsp. *anitratus*	Teicoplanin, cefepime	10 days	Cured

of the slow growth of the isolates. Recent advances in testing methods, particularly MALDI–TOF MS and 16S rRNA sequencing, have made it possible to identify *A. neuii* subsp. accurately in clinical samples[18]. Indeed, in this study, we were able to identify *A. anitratus* accurately using MALDI–TOF MS and 16S rRNA sequencing. In clinical practice, MALDI–TOF MS is useful because it is fast and accurate.

Among 762 commensal gram-positive rods routinely isolated from patient samples, the most frequently detected isolates were *Cutibacterium acnes* (33%), *Corynebacterium striatum* (20%), and *A. neuii* (8%), followed by multiple *Corynebacterium* species[10]. However, cases of bloodstream infections caused by *A. neuii* have rarely been reported[8]. A total of 10 case reports of *A. neuii* bloodstream infections that provided clinical information have been described in the literature, and the clinical characteristics of these patients, including the case described here, are summarized in Table 2[1,3,4,6–8,12,14,20].

The most common source of primary bloodstream infection was infective endocarditis (30%, 3/10 cases). Two neonatal cases of suspected intrauterine infection have also been reported. As some reports have not identified *A. neuii* at the subspecies level, the prevalence of *Actinomyces neuii* subsp. *anitratus* remains unclear. However, a previous study reported 3 cases (8%, 3/36) of bloodstream infection with *Actinomyces neuii* subsp. *neuii* and 4 cases (13%, 4/31) of bloodstream infection with *Actinomyces neuii* subsp. *anitratus* among 67 patients with *Actinomyces neuii* infection[6].

In this case, culture of the removed CV port was not performed, and the culture of the catheter tip yielded negative results. However, physical examination revealed a suspected CV port infection, which progressed to septic shock, and a culture of all other specimens yielded negative results. Therefore, although *A. neuii* subsp. *anitratus* was only cultured from the blood, we finally diagnosed the patient with bloodstream infection caused by *A. neuii* subsp. *anitratus* due to CV port infection, and not contamination of the blood culture. The antimicrobial susceptibility pattern of *A. neuii* corresponds to that of other *Actinomyces* spp[17]. Therefore, beta-lactam antimicrobial agents, such as penicillin or ampicillin, are usually used for treatment.

In this case, the patient's condition was severe at admission, and a Gram stain of the blood culture sample yielded findings that were consistent with *Corynebac≠*

terium spp. In addition, the identification and antimicrobial susceptibility tests took approximately six days. Therefore, empiric TEIC and CFPM were continued after identification of *A. neuii* subsp. *anitratus*, and so antimicrobial treatment was administered for a total of 10 days after CV port removal.

In conclusion, we report a case of bloodstream infection caused by *A. neuii* subsp. *anitratus* due to CV port infection. Mass spectrometry and 16S rRNA sequencing were found to be more useful for the identification of *A. neuii* subsp. *anitratus* compared to phenotypic identification. Further research on *A. neuii* subsp. *anitratus* infections is required to avoid delayed or missed diagnoses.

Declaration of interest

None.

Consent

No sensitive data nor images were used in the elaboration of this manuscript.

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Author contributions

Toshinori Hara: Conceptualization, Methodology, Data curation, Writing - original draft, Writing - Review & Editing, Visualization, Hiroki Kitagawa: Writing - Review & Editing, Visualization, Toshiki Kajihara: Resources, Writing- Review & Editing , Yumiko Koba: Resources, Kayoko Tadera: Resources, Rie Nagaoka: Resources, Ohta Shiho: Resources, Yusuke Nakaoka: Resources, Toshihito Nomura: Writing - Review & Editing, Keitaro Omori: Writing - Review & Editing, Norifumi Shigemoto: Writing - Review & Editing, Shinsuke Sasada: Writing - Review & Editing, Kohei Ota: Resources, Seiya Kashiyama: Writing- Review & Editing, Michiya Yokozaki: Writing- Review & Editing, Nobuaki Shime: Writing- Review & Editing, Supervision, Hiroki Ohge: Writing- Review & Editing, Supervision

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