Editorial

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# Toward a Refined Framework for *Diploscapter* Species Identification

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The study deals with the intricate and underexplored issue of species identification within the genus *Diploscapter*, focusing particularly on *D. coronatus* — a nematode with rare yet notable presence in human specimens[1]. The authors combine classical morphological observation with molecular approaches, namely SSU rRNA and Hsp90 gene sequencing, to evaluate a nematode isolated from a patient with Henoch-Schönlein purpura. While the study offers a snapshot of the complexities involved in identifying *Diploscapter* species, it also highlights broader challenges in nematode taxonomy that warrant further exploration.

One of the most significant insights emerging from this work is the discordance between morphological identification and molecular phylogeny. Despite morphological alignment with *D. coronatus*, the Hsp90-based phylogenetic placement within the *D. lycostoma* cluster reveals potential limitations in both gene-targeted analysis and the current state of the genetic database. This finding reflects a common issue in parasitology: using only one gene or morphometric approaches often isn't enough to clearly identify species, especially in groups that look very similar and have little genetic information available. The authors point out the lack of comprehensive reference data for *Diploscapter* species. So far, only three species (including the newly described *D. formicidae*) have been well-characterized in genetic databases. This lack of information makes it hard not only to correctly identify species but also to understand which hosts they inhabit and how they have evolved. Furthermore, one sample can be placed into two different species groups depending on which gene (SSU rRNA or Hsp90) is used. This shows that using just one gene may not be reliable, and studying many genes or even the whole genome is important — especially when the species might affect human health.

Notably, while males are generally thought to be absent in the genus *Diploscapter*, the study reports their presence. This rare discovery is important. If more examples of male reproductive features can be found and studied in *Diploscapter*, it could lead to new ways of identifying species — not just by looking at genes, but also by including differences in how they reproduce. Another important point is the idea that the host an organism lives with, along with its environment, might influence how related species are grouped in evolutionary trees. Some *Diploscapter* species, like *D. formicidae* and *D. lycostoma*, have been found in ants. This could suggest a hidden pattern of co-evolution between these nematodes and their hosts, which we haven't fully understood yet because there haven't been many studies or reports. Human infections are very rare, so each case is especially valuable. The authors' report of a specimen found in a human is an important finding. These findings serve as both a warning and a reminder that we need to do more. Accurately identifying nematode species — especially in groups like *Diploscapter* — requires strong and varied methods. To move forward, we need to expand genetic databases, use physical characteristics more consistently, and make genetic testing easier to access in parasite research and diagnosis.

This study is highly valuable for its careful and clear investigation of a topic that is important to both biology and medicine. The study contributes important new data and raises intriguing questions for future research: How many different *Diploscapter* species are there really? Can the type of host they live in or the shape of male features

help us tell them apart? And how can we make sense of differences between genetic data and physical traits?

This study shows that identifying species is not just a technical task — it also challenges how we understand and interpret what we see, especially with the limits of the tools we currently have. In this way, the research takes an important step toward a deeper and more complete understanding of a group of nematodes that we still know little about, but that are important in biology.

**References**

1) Watthanakulpanich D, Anantaphruti MT, Maipanich W. Diploscapter coronata infection in Thailand: report of the first case. Southeast Asian J Trop Med Public Health. 2005; 36 (Suppl 4) : 99-101.

*Short Communication*

# Analysis of food allergy history of patients who developed mild allergic transfusion reactions

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**ABSTRACT**

We examined the history of food allergy (FA) in patients at the University of Yamanashi Hospital who were hospitalized from 2019 to 2021 and developed allergic transfusion reactions (ATRs). One hundred fifty-seven patients developed mild ATRs, among which 22 patients had a history of FA. Out of the 157 patients, 30 patients received red blood cell (RBC) transfusion, among which 20.0% (6/30) had FA. Thirty patients received fresh frozen plasma (FFP) transfusion, and 23.3% (7/30) of them had FA. Ninety-seven patients received platelet concentrate (PC) transfusion, and 9.3% (9/97) of them had FA. The FA history-positive rate was 21.7% (13/60) in patients who developed mild ATRs after RBC or FFP transfusion, which was significantly higher than that (11.1%, 4,380/39,473) in all patients hospitalized and that in patients without any adverse transfusion reactions (3.1%) during the same periods. We suggest the possibility that allergic food antigens included in blood products might partly (maximally 10%) be involved in the occurrence of mild ATRs after RBC or FFP transfusion. Despite the higher incidence of ATRs after PC transfusion, the involvement of FA was not demonstrated probably because more multimodal factors are implicated in ATRs after PC transfusion.

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**Key Words**
allergic transfusion reactions, food allergy history, allergic predisposition

## I. Introduction

Transfusion adverse reactions are classified as hemolytic side effects, non-hemolytic side effects, and infections. Non-hemolytic transfusion reactions are classified as allergic transfusion reaction (ATR), febrile nonhemolytic transfusion reaction (FNHTR), transfusion-related acute lung injury (TRALI), transfusion-associated circulatory overload (TACO), hypotension, bacterial sepsis, and others[1),2)].

Among them, allergy (allergic transfusion reactions; ATRs) is the most frequent and mild-symptomatic transfusion reaction[1)]. An allergy occurs if the immune response shows excessive reactions to eliminate foreign antigens. However, most of the causes of allergy to transfusions have not been elucidated. The only known cause

of allergy to blood transfusions is in patients deficient of plasma proteins (IgA or haptoglobin), who produce antibodies to these proteins due to previous blood transfusions[3), 4)]. Food allergy (FA) is also an allergy elicited through antigen-specific immunological mechanisms induced by food.

In this study, we investigated the association between allergy after blood transfusion and a history of food allergy in patients who underwent blood transfusion at the University of Yamanashi Hospital.

## II. Methods and subjects

This study was judged by the Ethics Committee of the University of Yamanashi School of Medicine as not requiring ethical review. For a period of three years from January 2019 to December 2021, we studied patients who underwent blood transfusion and developed transfusion adverse reactions at the University of Yamanashi Hospital. During this period, 14,146 red blood cell (RBC) products, 7,896 fresh frozen plasma (FFP) products, and 6,595 concentrated platelet (PC) products were transfused to the patients. All blood products including RBC, FFP, and PC were transfused based on Guideline for the use of RBC products[5)], FFP[6)], and PC[7)], respectively. As a result, 247 patients developed transfusion adverse reactions. Some patients developed the adverse reactions following transfusion of more than one product (i.e., FFP, RBCs, and platelets). We observed 157 patients who developed allergy after transfusion of a single product.

Classification of adverse transfusion reactions was determined by a physician based on the guideline of the Japan Society on Transfusion Medicine and Cell Therapy as shown in Supplemental **Table 1**[8)]. "Allergic adverse reactions (ATRs)" refer to adverse transfusion reactions with[4)] pruritus,[5)] flushing,[6)] urticaria. When patients are served allergen-free meals during hospitalization, they are assumed as patients with food allergies.

We compared the FA history of these 157 patients who developed allergy reactions with that of all hospitalized patients (39,473 patients). For this, the statistical method chi-square test was used ($p < 0.05$ was considered to show a significant difference).

The all-inpatient data including ages, gender, and their afflicted disease were from the hospital statistics of University of Yamanashi Hospital shown in the homepage (https://www.hosp.yamanashi.ac.jp/wp-content/uploads/2022/12/17d3e528c13ac6d071ec4dbf0a9b801d-1.pdf).

## III. Results

### Breakdown of adverse blood transfusion reactions

Among 247 patients who developed transfusion reactions, no hemolytic adverse reactions were observed, and all were non-hemolytic. Excluding patients who underwent transfusion of multiple products, 200 patients (339 cases) developed adverse transfusion reactions (**Table 1A**). Among these 339 cases, 0.44% (62 cases) developed adverse reactions after RBC transfusion, 0.72% (57 cases) after FFP transfusion, and 3.34% (220 cases) after PC transfusion. Furthermore, among the 339 cas-

**Table 1A** Types of Adverse Blood Transfusion Reactions

| | No. of cases | No. of patients*1 | No. of patients by blood transfusion products | | |
|---|---|---|---|---|---|
| | | | RBC | FFP | PC |
| Non-hemolytic blood transfusion reactions*2 | 339 | 200 | 57 (62 cases) | 37 (57 cases) | 106 (220 cases) |
| of the above minor reactions | 330 | 193 | 55 (96.5%) | 33 (89.2%) | 105 (99.1%) |
| of the above allergy | 286 | 157 | 30 (54.4%) | 30 (90.9%) | 97 (92.4%) |
| of the above allergy with FA | 58 | 22 (14.0%) | 6/30 (20.0%) | 7/30 (23.3%) | 9 /97 (9.3%) |
| | | | 13/60 (21.7%) | | |

*1 Patients who had separate reactions with different blood products were counted as separate patients.
*2 Excluding compound blood product administration
RBC; red blood cells, FFP: fresh frozen plasma, PC; platelet concentrates, FA; food allergy

**Table 1B** Percentage of FA patients in patients without adverse transfusion reactions and in all in-patients (2019 – 21)

| | All patients | FA patients | Percentage |
|---|---|---|---|
| Patients without adverse transfusion reactions | 2830 | 89 | 3.1 % |
| All in-patients | 39473 | 4380 | 11.1% |

es (200 patients), 330 cases (193 patients) were mild. Among the mild 330 cases (193 patients), 286 cases (157 patients) were allergic (mild ATRs) (**Table 1A**).

**Association between FA history and adverse reactions to blood transfusion**

Among the 157 patients who developed allergy reactions after transfusion, 14.0% (22/157) have an FA history. Furthermore, among these 157 patients, 30 patients received RBC transfusion and 20.0% (6/30) of them had a history of FA. Thirty patients received FFP transfusion, and 23.3% (7/30) of them had a history of FA. Ninety-seven patients received PC transfusion, and 9.3% (9/97) of them also had a history of FA (**Table 1A**).

During the study period, the total number of hospitalized patients was 39,473, among which 11.1% (4,380/39,473) had an FA history (**Table 1B**). Compared with this, the percentage of patients with a history of FA in RBC transfused or FFP transfused patients (21.7%) was significantly higher ($p = 0.042$). FA history rate of the patients who underwent blood transfusion without any adverse transfusion reactions was also investigated (**Table 1B**), and was 3.1% (89/2830), much lower than the FA rate of the ATR patients with FFP/RBC transfusion (21/7%) ($p = 2.12 \times 10^{-13}$). On the other hand, there was no significant difference in the FA history rate between the PC transfused patients and all hospitalized patients.

**Table 2** shows a list of foods (the number of patients in parentheses) excluded from allergen-free meals during their hospitalization.

**Baseline characteristics**

Baseline characteristics of the patients with ATRs and all the hospitalized patients were shown in Supplemental **Figures 1 and 2**. Age structure in increments of 5 years of the ATR patients (157) and all inpatients from 2019 to 2021 are shown in Supplemental **Figure 1A, B**. we found no difference of the ratios of 0～4 years old patients between the 2 groups ($p = 0.981$, proportion z-test with Yates' continuous correction). Moreover, there is no statistical significance of all the patient age compositions between the 2 groups ($p = 0.999$, paired T test). Therefore, higher percentage of FA in the ATR patients is not because of higher percentage of infants.

As for gender, the male ratio was not different between in the ATR group and in the all-inpatient group ($p = 0.262$, proportion z-test with Yates' continuous correction) (**Supplemental Figure 1C**).

**Supplemental Figure 2** shows that disease compositions of the 2 groups are different from each other. Sixty-nine % of the ATR patients was malignant neoplasm patients, whereas only 31% in the all-inpatient group. As expected, most of malignant tumor in the ATR group is hematological tumor. This is followed by Hematologic disease except for neoplasm, which is included in Others due to only a limited percentage in the all inpatient group.

## IV. Discussion

Although ATRs are the most frequent transfusion-related adverse reactions, the search for the cause is not usually carried out in mild cases, and thus the mechanism is not fully understood[9]. However, previous reports suggest that allergic constitution such as bronchial asthma, hay fever, atopic dermatitis, and food allergy are closely related to the development of ATRs[10)-12)].

The prevalence of FA in Japan is reported to be 4.0 - 16.7% in infancy, 1.3 - 4.5% in school-age children, and less than 5% after school age[13]. However, after the school age, infants with FA often find it possible to ingest the food they are allergic to. Ebisawa et al. reported that the cumulative food allergy incidence was 64.5% in 2-year-old children, 80.3% in 5-year-old children, and 90.1% in 10-year-old children based on a survey of 2,954 cases[14]. On the other hand, the precise percentage of those with a history of FA in the general population is unknown. In this study, by examining the number of patients who were served allergen-free meals, we found

**Table 2** A list of foods excluded from allergen-free meals during the hospitalization (the number of patients)

| | | |
|---|---|---|
| Fruits (5) | Soybeans (1) | Fish (1) |
| Peach (1) | Soy milk (1) | Mackerel (4) |
| Grapefruits (5) | Fermented soybeans (1) | Shrimps, crabs (4) |
| fresh vegetables (1) | Nuts (1) | |
| Tomato (2) | Egg white (2) | |
| Mushrooms (1) | dairy products (2) | |
| Bracken (1) | Cow milk (2) | |

*With duplicate entries

that approximately 10% of inpatients at the University of Yamanashi Hospital have a history of FA.

There is no difference in the infant ratios between the ATR patient and all inpatient groups (**Supplemental Figure 1A, B**). Therefore, higher percentage of FA in the ATR patients is not because of higher percentage of infants.

The FA history-positive rate was 3.1% (89/2830) in patients who did not develop adverse transfusion effects after blood transfusion, which was significantly lower than that in patients who developed ATRs after RBC or FFP transfusion (21.7%, 13/60) ($p = 2.12 \times 10^{-13}$). This rate (3.1%) is also significantly lower than the ratio in all patients in (11.1%, 4,380/39,473) ($p = 3.88 \times 10^{-40}$). These findings further support that food allergy is at least one of the causes of ATR.

Despite allergen-free meals, if the patient remains sensitized, the allergic reaction may be triggered when the allergen in the food enters the body from blood donors through blood transfusion. However, there have only been a few reports on ATR, probably because of the difficulty in identifying the causative food. Previous case reports identified that blood recipients with a history of allergy to peanuts or shrimp suffered ATR and anaphylaxis due to the transmission via blood products of the antigens ingested by blood donors[15),16)]. In this study, we reported that the rate of FA history in patients who developed mild ATRs after receiving RBCs/FFP was significantly higher than that in other hospitalized patients. We suggest that some (up to 10%) of RBC- and FFP-related ATRs may have been induced by food antigens in the donor serum. On the other hand, the pathogenic mechanisms of PC-related ATRs, which have a very high incidence, are more diverse (e.g., involvement of blood cell-derived cytokines). The involvement of food antigens was relatively low.

Ebisawa et al. reported that most frequent causative foods for immediate food allergy were hen's egg (39.0%), cow's milk (21.8%), and wheat (1.7%)[14)]. However, in this study the most frequent causative foods were grapefruits, shrimps/crabs, and mackerel (**Table 2**), which may be due to differences in age groups.

In conclusion, we found that the FA history-positive rate was 21.7% in patients who developed mild ATRs after RBC/FFP transfusion, which was significantly higher than that in all hospitalized patients (11.1%) and that in hospitalized patients without any adverse transfusion reactions (3.1%). We suggest the possibility that allergic food antigens included in blood products might partly be involved in the occurrence of mild ATRs, although a large-scale study is required to clarify the role of FA in the development of mild ATRs.

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**References**

1) Yanagisawa R. Preventing adverse reactions in pediatric transfusions using washed platelet concentrate. Pediat Int. 2021; 63 (4): 391-403. doi: 10.1111/ped.14572.
2) Moncharmont P. Adverse transfusion reactions in transfused children. Transfus Clin Biol. 2019; 26 (4): 329-35. doi: 10.1016/j.tracli.2019.08.002.
3) Robitaille N, Delage G, Long A, et al. Allergic transfusion reactions from blood components donated by IgA-deficient donors with and without anti-IgA: a comparative retrospective study. Vox Sang. 2010; 99 (2): 136-41. doi: 10.1111/j.1423-0410.2010.01326.x.
4) Anani W, Triulzi D, Yazer MH, et al. Relative IgA-deficient recipients have an increased risk of severe allergic transfusion reactions. Vox Sang. 2014; 107 (4): 389-92. doi: 10.1111/vox.12192.
5) Yonemura Y, Matsumoto M, Inada E, et al. Guideline for the use of red blood cell products based on scientific evidence (revision 2nd edition). Japanese Journal of Transfusion and Cell Therapy. 2018; 64 (6): 688-99.
6) Matsushita T, Hasegawa Y, Tamai Y, et al. Guideline for the use of fresh frozen plasma based on scientific evidence (revised second version). Japanese Journal of Transfusion and Cell Therapy. 2019; 65 (3): 525-37.
7) Takami A, Matsushita T, Ogata M, et al. Guideline for the use of platelet transfusion concentrates based on scientific evidence: update 2019. Japanese Journal of Transfusion and Cell Therapy. 2019; 65 (3): 544-61.

8) Kato H. Signs and symptoms of transfusion reactions. In: Japanese Society of Blood Transfusion and Cell Therapy Task Force for the preparation of a revised practical guide for management of transfusion reactions, editor. The practical guide for management of transfusion reactions. Version 1.0. Tokyo: The Japan Society of Transfusion Medicine and Cell Therapy. 2014: P.24-8

9) Yasui K, Matsuyama N, Takihara Y, et al. New insights into allergic transfusion reactions and their causal relationships, pathogenesis, and prevention. Transfusion. 2020; 60 (7) : 1590-601. doi: 10.1111/trf.15845.

10) Savage WJ, Hamilton RG, Tobian AAR, et al. Defining risk factors and presentations of allergic reactions to platelet transfusion. J Allergy Clin. Immunol. 2014; 133 (6) : 1772-5. e9. doi: 10.1016/j.jaci.2014.03.030.

11) Yanagisawa R, Ishimine N, Komori K, et al. Relationship between allergic transfusion reactions and allergic predisposition among pediatric patients with hematological/oncological disease. Transfusion. 2022; 62 (5) : 1035-44. doi: 10.1111/trf.16855.

12) Ide Y, Yanagisawa R, Kobayashi J, et al. Relationship between allergic sensitisation-associated single-nucleotide polymorphisms and allergic transfusion reactions and febrile non-haemolytic transfusion reactions in paediatric cases. Blood Transfus. 2022; 20 (2) : 94-102. doi: 10.2450/2021.0230-20.

13) Matsubara Y, Ae R, Ohya Y, et al. [Estimated number of patients with food allergy in Japan: The present status and issues regarding epidemiological investigation]. Arerugi. 2018; 67 (6) : 767-73. doi: 10.15036/arerugi.67.767.

14) Ebisawa M, Ito K, Fujisawa T. Japanese guidelines for food allergy 2020. Allergol Int. 2020; 69 (3) : 370-86. doi: 10.1016/j.alit.2020.03.004.

15) Jacobs JFM, Baumert JL, Brons PP, et al. Anaphylaxis from passive transfer of peanut allergen in a blood product. N Engl J Med. 2011; 364 (20) : 1981-2. doi: 10.1056/NEJMc1101692.

16) Gao L, Sha Y, Yuan K, et al. Allergic transfusion reaction caused by the shrimp allergen of donor blood: a case report. Transfus Apher Sci. 2014; 50 (1) : 68-70. doi: 10.1016/j.transci.2013.09.016.

*Case Report*

# Unexpected fungal aortitis leading to aortopulmonary fistula: an autopsy case

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**ABSTRACT**

We present a possibly unique autopsy case of fatal aortopulmonary fistula associated with subclinical infectious aortitis in an elderly man. After 6-week antifungal therapy for candidiasis in his eyes accompanying leakage from anastomoses following gastrectomy, β-D-glucan levels remained elevated despite negative blood cultures. Six weeks after completion of the antifungal therapy, he was re-hospitalized for hematochezia possibly due to bleeding associated with the anastomosis sites, and arterial embolization was successfully performed. However, about three weeks after the embolization, he died of hemorrhagic shock due to sudden bloody "vomiting" . Autopsy revealed no anastomosis-related bleeding but an unexpected non-aneurysmal *Candidal* aortitis-related aortopulmonary fistula. This had caused massive fatal hemorrhage into the lung parenchyma and resultant bloody "vomiting". We believe that the long-standing elevated β-D-glucan level, even with negative blood culture results, may indicate subclinical fungal aortitis. This may have directly contributed to our patient's death, although such a fatal event may be rare.

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**Key Words**

Aortopulmonary fistula, β-D-glucan; candidiasis, infectious aortitis

## I. Introduction

Infectious aortitis (IA) is an uncommon disease causing mycotic or infectious aneurysms, but it is also known to provoke life-threatening aortic rupture or perforation due to infection-related aortic wall fragility even without aneurysm formation.[1)-7)] Common causes of primary aortic infection include bacterial pathogens such as *Staphylococcus*, *Streptococcus*, and *Salmonella* species.[1)-5)] Fungal pathogens -- such as *Candida, Aspergillus*, *Cryptococcus,* or *Histoplasma* -- may cause aortic infection, too, albeit infrequently.[2)-5),8)-13)] From the therapeutic point of view, early detection and diagnosis of IA are clinically required. However, the antemortem diagnosis of IA is challenging in cases without aneurysmal changes because patients with non-aneurysmal IA are often asymptomatic and lack specific manifestations.[1)-3),6),7)] Here, we describe the unique clinicopathological features of a case (an elderly

man) with non-aneurysmal IA. Autopsy revealed a fungal aortitis-associated aortopulmonary fistula contributing to massive pulmonary hemorrhage. We also reviewed the laboratory data obtained in the present case for findings that might aid the detection of subclinical fungal aortitis.

## II. Case presentation

Patient: A 79-year-old man

Past history: Diabetes mellitus, gastric cancer, rectal cancer

The patient developed leakages at two intestinal sites with localized abscess-formation during the post-operative course of laparoscopic total gastrectomy for gastric cancer. Culture from the drainage fluid collected on post-operative Day-3 and blood cultures on post-operative Day-12 revealed positive results for *Candida albicans*. *Candida* infection-related endophthalmitis was also diagnosed. A six-week antifungal therapy regimen was instituted. This led to shrinkage of the abscess cavity near the leakage site, and thereafter blood cultures were negative for *Candida*. The serum (1 → 3)-β-D-glucan (BDG) level, which was 757 pg/mL (normal range: ≤ 20 pg/mL) at the time of the first positive blood culture (post-operative Day-13), decreased to 133 pg/mL after completion of the antifungal therapy (post-operative Day-86). The patient was considered to be recovered and was discharged 3 months after the gastrectomy, but he was re-hospitalized for hematochezia 10 days after discharge. Laboratory data obtained at re-hospitalization showed anemia (Hb 4.5 g/dL), a normal white blood-cell count (4,900/μL), and an elevated serum C-reactive protein (CRP) level (4.4 mg/dL). On the fourth day of re-hospitalization, the serum CRP level had increased to 14.9 mg/dL, and the serum BDG remained positive (108 pg/mL). Empiric antibiotic therapy for possible bacterial infection was initiated, and endoscopy confirmed fresh bleeding near the previous anastomosis leakage site. The responsible jejunal branch artery was successfully embolized. Cultures from the central venous catheter tip and urine were negative. No blood culture was obtained during the second hospitalization. About three weeks after the embolization (4 months after the gastrectomy), the patient died of massive hemorrhagic "vomiting", which was clinically suggested to be attributable to recurrent anastomosis-related bleeding. Clinical course and laboratory data are summarized in **Table 1**.

Autopsy revealed no bleeding at anastomosis sites or other enteric regions, although fresh blood clots were present in the oral cavity, esophagus, and trachea. The thoracic aortic wall was severely atherosclerotic but without obvious aneurysmal dilatation (**Figure 1a**), and it was firmly adherent to the left lung surface (**Figure 1b-c**). No left hemothorax was found. Histologically, the aortic wall structure, where it was tightly adherent to the left lung, was not only fibrously disrupted with scattered loss of elastic fibers, but also showed a transmural neutrophilia with *Candida*-like fungal growth and fistula formation (**Figure 2a-d; 3a-b**). A massive blood accumulation was found within the left lung parenchyma, resulting from aortic hemorrhage caused by this aortopulmonary fistula. (**Figure 3c**). Blood influx to the contralateral lung was also found, and it had probably occurred via the airway from the left lung. We concluded fungal aortitis-related aortopulmonary fistula causing pulmonary hemorrhage. Autopsy disclosed fungal growth in both kidneys, but it failed to reveal a similar fungal infection in the aortic wall other than in the adherent thoracic aorta.

Retrospective evaluation of computed tomography (CT)

**Table 1** Summary of clinical course and data.

| Post-operative day | 0* | 3 † | 12 | 13 | 14 | 16 | 18 | 23 | 86 | 101 ‡ | 102 § | 104 | 113 | 120 ¶ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WBC (× 1,000/μL) | 5.0 | 13.7 | 7.0 | 7.8 | 9.6 | 8.6 | 9.9 | 7.2 | 4.7 | 4.9 | 8.2 | 5.2 | 5.8 | 6.7 |
| CRP (mg/dL) | 0.4 | 26.5 | 13.6 | 16.3 | 25.2 | 17.4 | 10.8 | 11.5 | 4.8 | 4.4 | 3.8 | 14.9 | 14.8 | 12.1 |
| Hb (g/dL) | 11.9 | 10.0 | 10.7 | 9.0 | 9.7 | 8.2 | 9.1 | 7.6 | 9.0 | 4.5 | 7.2 | 8.9 | 9.1 | 7.9 |
| BDG (pg/mL) | | | | 757 | | | | 945 | 133 | | | 108 | | |
| Culture results for *Candida* | | + (d) | + (b) | | | + (d)<br>- (b) | | - (d)<br>- (b) | | | | - (c) | - (u) | |
| Antifungal medication | | | | | MCFG (~Day15) | CPFG (~Day17) | L-AMB (~Day59) | | | | | | | |

Important events occurring on certain days are indicated as follows: Day 0*: gastrectomy. Day 3 †: anastomosis leakage.
Day 101 ‡: The patient was re-admitted and IVR (interventional radiology) was administered. Day 102 §: 2nd IVR.
Day 120 ¶: Patient death.
Abbreviations: WBC, white blood cell count; CRP, C-reactive protein; Hb, hemoglobin; BDG, β-D-glucan;
+ (d), positive in drainage fluid; + (b), positive in blood; - (b), negative in blood; - (d) negative in drainage fluid;
- (c) negative at central venous catheter tip; - (u) negative in urine; MCFG, Micafungin; CPFG, Caspofungin;
L-AMB, Liposomal Amphotericin B.

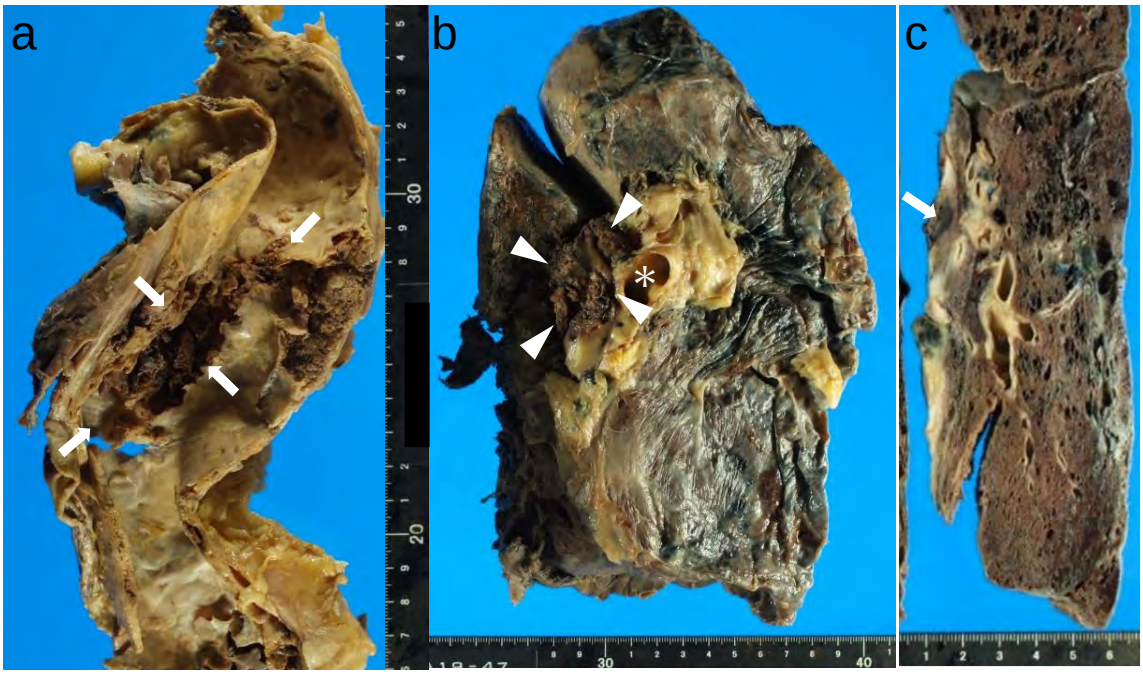


**Figure 1** Gross appearance of the descending aorta a) and the left lung b-c).

**a**) Severely atherosclerotic thoracic descending aorta without distinct aneurysmal features. Aortic wall was tightly adherent to the left lung, with its detachment from the lung causing a partial tissue defect (arrows).

**b**) Mediastinal side of the left lung showing partial aortic wall (arrowheads) tightly attached to the lung behind the left main bronchus (asterisk).

**c**) On the cut surface, emphysematous lung changes and dark brown discoloration are present, along with hematoma formation at the site of aortic adhesion (arrow).

revealed progressive enlargement of a shadow outside the calcified atherosclerotic thoracic aorta with no apparent aneurysmal changes (**Figure 4a-d**).

## III. Discussion

Cases with IA are usually at risk of aortic rupture/perforation via aneurysmal changes, but their aorta infrequently ruptures without such changes.[1)-7)] Non-aneurysmal IAs may develop a fistula to the esophagus, duodenum, or jejunum,[2)-4)] and Orend *et al*[7)] noted that non-aneurysmal IAs had perforated in 8% of 54 cases of aortic rupture. In the current case a non-aneurysmal IA directly penetrated the left lung, resulting in a massive hemorrhage. The adherent aortic wall and medial elastic fibers meshwork were vaguely disrupted by a fungal infection. These findings suggest that the fragility of the infected aortic wall may have played a critical role in the development of the fatal aortopulmonary fistula. In addition, at the fistula site there were severe atherosclerotic changes. These changes may have provoked weakening of the aortic wall and the formation of a close adhesion to the paraaortic structures, which can sometimes result in a fistula.[14)] Moreover, the adhesion of *Candida* species can be triggered by severe atherosclerosis-related intimal ulceration and/or coarseness.[3)]

An IA-related aortopulmonary fistula is rare, and our review of the literature disclosed only five cases for which detailed clinicopathological findings were published (**Table 2**).[15)-18)] Among these, four cases (all cases with description) were accompanied by aneurysmal or pseudo-aneurysmal aortic changes. The current case lacked any obvious IA-related aneurysmal changes. The fistula occurred between the aorta and the left lung in all 6 cases, possibly due not only to its anatomical proximity to the descending aorta, but also to this being the favored IA-related fragile site with or without aneurysmal changes. Regarding the pathogen of IA, in all four of the above cases with description, the IA was caused by bacteria, while the present case alone was infected by fungus. BDG was not measured in any of the previous cases. Three patients died of their disease, suggesting that it is often a fatal condition. The other 2 patients were alive after surgery, and in one of these the fistula was detected fortunately by bronchoscopy. The remaining case was diagnosed as a fistula by CT scanning for evaluation of massive bloody "vomiting". Accordingly, aggressive examination at the time of hemoptysis or massive bloody "vomiting" seems likely to lead to the early detection of an aortopulmonary fistula and contribute to a good prognosis. However, hemoptysis was noted in only half of the

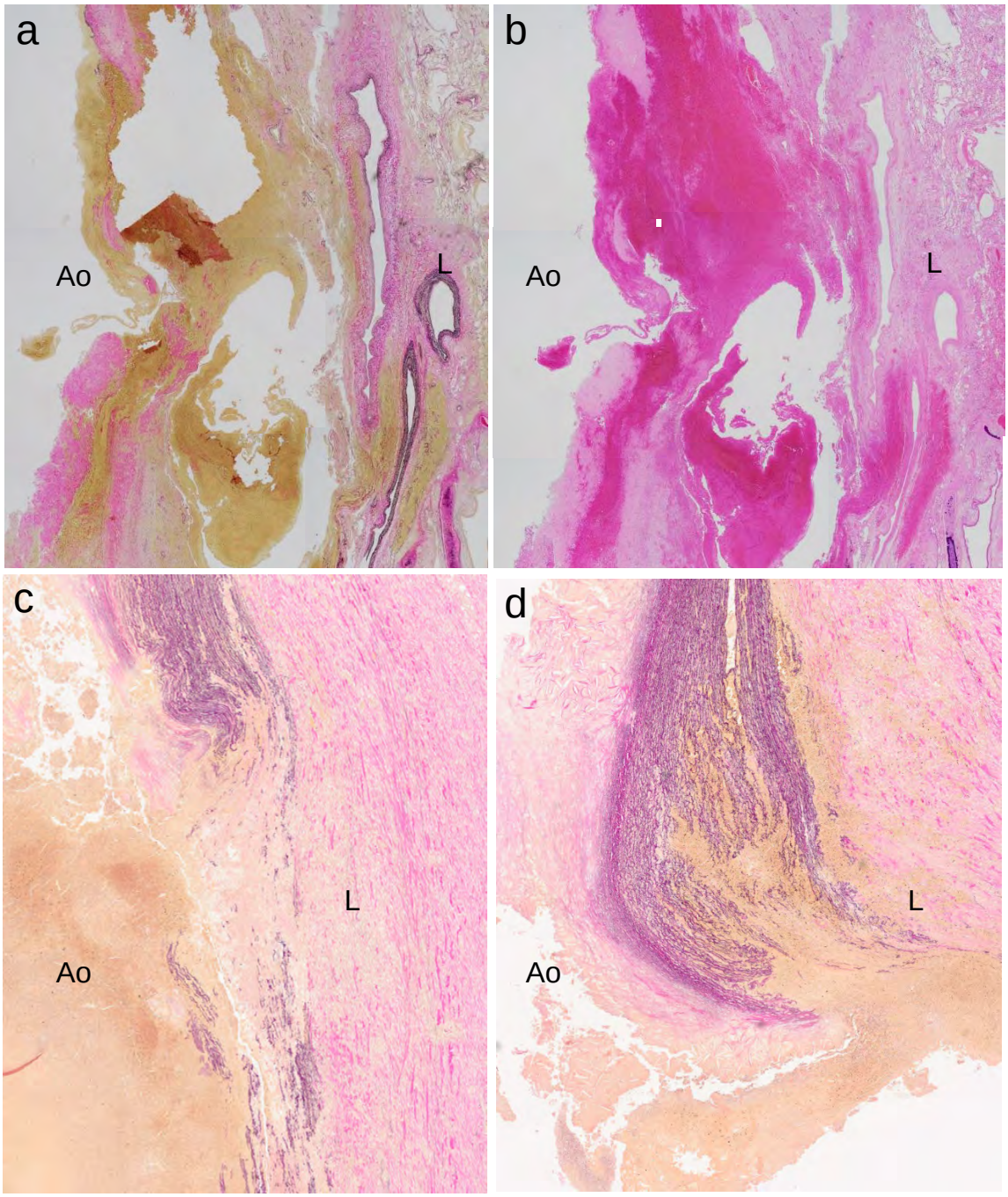


**Figure 2**

**a, b**) In the panoramic image of the aortopulmonary adhesion (a: EVG, b: HE), aortic wall rupture with hemorrhage can be observed.

**c, d**) Aortopulmonary fistula (EVG). c) The aortic wall shows degeneration, loss of elastic fibers, and d) eventual rupture due to complete tear.

**Ao**: aortic lumen, L: lung, EVG: elastica van Gieson stain, HE: hematoxylin eosin stain

aortopulmonary fistula cases, although this complaint may have been clinically misdiagnosed as enteric bleeding. Thus, the antemortem diagnosis of aortopulmonary fistula remains challenging, and is based chiefly on the patients' complaints. The absence of a hemothorax may play a role in an aortopulmonary fistula being difficult to recognize.

*Candida* infection can occur after esophageal surgery and is significantly associated with esophageal anastomosis leakage.[19] For establishing a diagnosis of invasive *Candida* infection, BDG testing is useful. BDG is a major fungal cell-wall polysaccharide and its measurement is based on the G-test, which has the same origin as the Limulus Amebocyte Lysate cascade reaction.[20)21)] In this

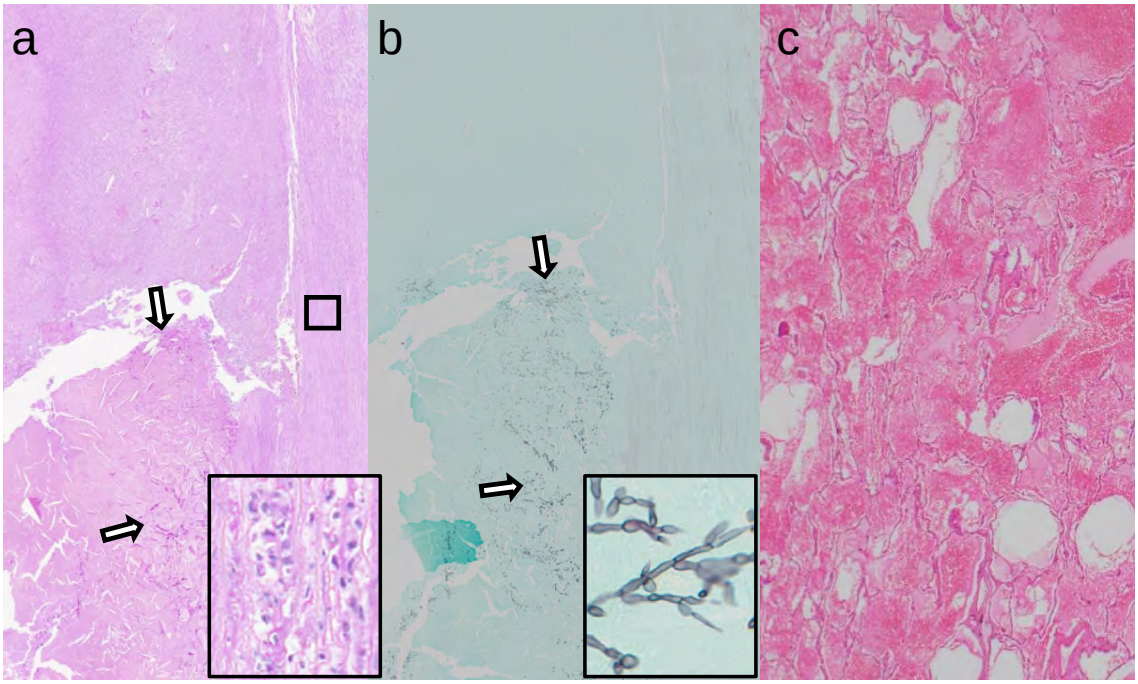


**Figure 3**

**a**) The panoramic image of the infected aorta shows diffuse inflammation and proliferations of *Candida*-like fungi in the atherosclerotic plaque (arrows). In the magnified view of the aortic wall shown in the inset, a marked infiltration of neutrophils can be observed (Periodic Acid-Schiff stain).

**b**) *Candida*-like fungi are highlighted particularly in the atherosclerotic plaque (arrows) (Grocott stain). In the inset, characteristic branching *Candida*-like hyphae can be observed.

**c**) In low magnification image of the peripheral left lung, diffuse accumulation of blood is observed in the alveolar space.

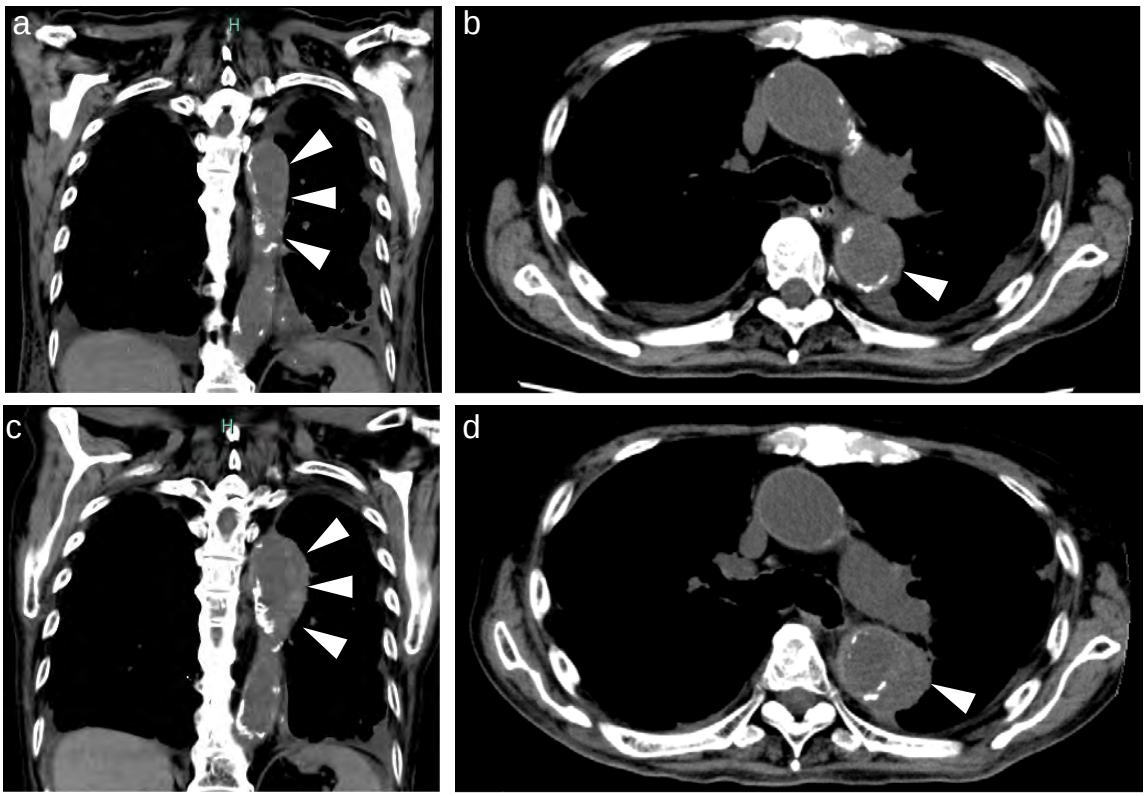


**Figure 4**

CT images obtained at the onset of candidiasis (**a**, **b**), and at 19 days before death (**c**, **d**). An obvious periaortic shadow (arrowheads) is observed in **c**) and **d**) versus **a**) and **b**).

**Table 2** Clinicopathological features of infectious aortitis-related aortopulmonary fistula with or without aneurysmal changes.

| | Case 1[15] | Case 2[15] | Case 3[16] | Case 4[17] | Case 5[18] | Present case |
|---|---|---|---|---|---|---|
| Age (yrs)/gender | 80/F | 85/F | 25/M | 59/F | 40/F | 79/M |
| Chief complaint | Unknown | Unknown | Toothache | Massive bloody vomiting | Bloody vomiting* | Bloody vomiting† |
| Immunocompromised state or antecedent condition | Unknown | Unknown | Ludwig angina and mediastinitis | Advanced hepatic alcoholic liver cirrhosis, DM | Unknown | DM, Anastomosis insufficiency after gastrectomy |
| Diagnosis of IA | Autopsy | Autopsy | Surgery | CT scan | Thoracic exploration | Autopsy |
| Diagnosis of fistula | Autopsy | Autopsy | Bronchoscopy‡ and subsequent surgery | CT scan | Bronchoscopy‡ and subsequent surgery | Autopsy |
| Hemoptysis | Unknown | Unknown | Present | Present | Present* | Present† |
| Pathogen of IA | Cocci (details unknown) | Unknown | *K pneumoniae, P. aeruginosa, H. aphrophilus, S. epidermis* | *Streptococcus agalactiae* | Acid fast bacilli | *Candida albicans* |
| Aortic dilatation and/or aneurysm associated with fistula | Present (pseudoaneurysm) | Present (pseudoaneurysm) | Unknown | Present (saccular aneurysm) | Present (saccular aneurysm) | Absent |
| Hemothorax | Absent | Absent | Unknown§ | Unknown | Absent | Absent |
| Location of fistula | DA to LL | DA to LL | DA to LL (lower lobe) | DA to LL | DA to LL (lower lobe) | DA to LL (lower lobe) |
| Outcome | DOD | DOD | Alive after surgery | Alive after surgery | DOD | DOD |

Abbreviations: CT, computed tomography; DA, descending aorta; DM, diabetes mellitus; DOD, dead of disease (aortopulmonary fistula); F, female; *H*, *Hemophilus*; IA, infectious aortitis; *K*, *Klebsiella*; LL; left lung; M, man; *P*, *Pseudomonas*; *S*, *Staphylococcus;* yrs, years.

* This hemorrhage was clinically misdiagnosed as duodenal ulcer-related bloody vomiting.

† This hemorrhage was clinically misdiagnosed as anastomosis leakage-related bloody vomiting.

‡ Bronchoscopic examination for evaluation of hemoptysis.

§ Purulent pleural effusion in the left thoracic cavity.

procedure, BDG activates Factor G, a serine protease zymogen found in the amebocytes of horseshoe crabs (Limulus). Activated Factor G then initiates a cascade of enzymatic reactions, and this results in the formation of an insoluble chromogenic substrate, which is measured to quantify BDG levels by either turbidimetric time analysis or synthetic substrate methods.[22] In our review of the laboratory data obtained in the current case, a negative blood culture was confirmed during the initial antifungal therapy, but the serum BDG levels were persistently elevated. Retrospective viewing of CT images revealed a progressive periaortic attenuated shadow outside the calcified aortic wall, which is considered one of the diagnostic imaging clues for infectious aortitis.[3,23] These findings suggest that a persistent deep-seated *Candida* infection provoked the aortitis in the present case. Indeed, fungal IA may be present even if the cultures are negative.[24,25] For detecting invasive candidiasis, the sensitivity of antemortem blood cultures has been found to be as low as 38% (in an analysis of 13 studies),[25] whereas that of serum BDG is higher at 75% (in 11 studies),[26] and Nguyen *et al*[24] highlighted the superiority of BDG versus blood culture. Therefore, a persistent BDG elevation may be the most reliable evidence of a subclinical prolonged fungal infection. However, it is important to note that false-positive results can occur due to the presence of hemodialysis, human blood products (albumin, immunoglobulin, coagulation factors, plasma protein fractions), surgical gauze, antibiotics (such as piperacillin-tazobactam and ampicillin-clavulanate), or systemic bacterial infections,[25] while false-negative results may be observed in infections caused by Cryptococcus, zygomycetes, and *Blastomyces dermatitidis*.[20-22] If results do not match the clinical course, further examination using

nucleic acid amplification is recommended.[25]

In conclusion, we describe a rare autopsy case of a non-aneurysmal *Candida* aortitis-related fatal aortopulmonary fistula. A prolonged increase in the serum BDG level would seem to indicate the possibility of a deep fungal-infection event despite blood cultures being negative. Our case might also indicate an increased risk associated with fungal aortitis in severely atherosclerotic patients, although such cases may be rare.

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**Author contribution statement**

OT drafted the original manuscript. OT, SO and MS conducted the pathological examination. SY, MT, and AM prepared the histological images and collected the references with interpretation of the autopsy results. NY and HT provided detailed information regarding the clinical course and insights about the CT images, OT, MO, SO, SM, NY and HT discussed the clinicopathological relationship. SO and SM reviewed the manuscript draft and revised it critically for intellectual content. All authors have read and approved the final version of the manuscript.

**Conflict of interest**

None of the authors have any conflict of interest relevant to the content of this article.

**References**

1) Gornik HL, Creager MA. Aortitis. Circulation. 2008; 117 (23): 3039-51. doi: 10.1161/CIRCULATIONAHA.107.760686.

2) Journeau L, de la Chapelle M, Guimard T, et al. A strobe multicenter descriptive study of 55 infectious aortitis. Medicine (Baltimore). 2020; 99 (40): e22422. doi: 10.1097/MD.0000000000022422.

3) Molacek J, Treska V, Baxa J, et al. Acute conditions caused by infectious aortitis. Aorta (Stamford). 2014; 2 (3): 93-9. doi: 10.12945/j.aorta.2014.14-004.

4) Parkhurst GF, Dekcer JP. Bacterial aortitis and mycotic aneurysm of the aorta; a report of twelve cases. Am J Pathol. 1955; 31 (5): 821-35.

5) Sörelius K, Budtz-Lilly J, Mani K, et al. Systematic review of the management of mycotic aortic aneurysms. Eur J Vasc Endovasc Surg. 2019; 58 (3): 426-35. doi: 10.1016/j.ejvs.2019.05.004.

6) Sommerville RL, Allen EV, Edwards JE. Bland and infected arteriosclerotic abdominal aortic aneurysms: a clinicopathologic study. Medicine (Baltimore). 1959; 38: 207-21. doi: 10.1097/00005792-195909000-00001.

7) Orend KH, Storck M, Brandl R, et al. Non-aneurysmal suppurative aortic rupture. Eur J Vasc Endovasc Surg. 1995; 10 (1): 118-21. doi: 10.1016/s1078-.5884 (05) 80208-4.

8) Leavitt AD, Kauffman CA. Cryptococcal aortitis. Am J Med. 1988; 85 (1): 108-10. doi: 10.1016/0002-9343 (88) 90513-x.

9) Barry MC, Jackson N, Adeboysku D, et al. *Candida albicans* mycotic abdominal aortic aneurysm. Eur J Vasc Endovasc Surg. 1997; 13 (2): 237-9. doi: 10.1016/s1078-5884 (97) 80028-7.

10) Fillmore AJ, Valentine RJ. Surgical mortality in patients with infected aortic aneurysms. J Am Coll Surg. 2003; 196 (3): 435-41. doi: 10.1016/S1072-7515 (02) 01607-1.

11) Malouf JF, Chandrasekaran K, Orszulak TA. Mycotic aneurysms of the thoracic aorta: a diagnostic challenge. Am J Med. 2003; 115 (6): 489-96. doi: 10.1016/s0002-9343 (03) 00394-2.

12) Kwon HH. Infected thoracic aortic aneurysm concurrent with endophthalmitis by *Candida albicans*: a case report. Korean J Med Mycol. 2017; 22 (4): 172-7. doi: 10.17966/jmi.2017.22.4.172 [In Korean].

13) Gunawardena T, Godakandage M, Abeywickrama S, et al. Mycotic aortic aneurysm in a debilitated patient with compromised immunity; beware of Candida! J Vasc Bras. 2021; 20: e20210122. doi: 10.1590/1677-5449.210122.

14) Gulati A, Kapoor H, Donuru A, et al. M. Aortic fistulas: pathophysiologic features, imaging findings, and diagnostic pitfalls. Radiographics. 2021; 41 (5): 1335-51. doi: 10.1148/rg.2021210004.

15) Origuchi N, Esaki Y, Shigematsu H, et al. [A clinicopathological study of ruptured infectious aneurysms]. Nihon Ronen Igakkai Zasshi. 1995; 32 (8-9): 553-9. doi: 10.3143/geriatrics.32 (8-9) .553 [In Japanese].

16) Economopoulos GC, Scherzer HH, Gryboski WA. Successful management of mediastinitis, pleural empyema, and aortopulmonary fistula from odontogenic infection. Ann Thorac Surg. 1983; 35 (2): 184-7. doi: 10.1016/s0003-4975 (10) 61458-0.

17) Masuhara H, Watanabe Y, Fujii T, et al. Successful surgical repair of an infectious thoracic aortic pseudoaneurysm accompanied by aortobronchopulmonary fistula and advanced hepatic dysfunction without assisted circulation. Ann Thorac Cardiovasc Surg. 2010; 16 (1): 35-9.

18) De Prophetis N, Armatage HV, Triboletti ED. Rupture of tuberculous aortic aneurysm into lung. Ann Surg. 1959; 150 (6): 1046-51. doi: 10.1097/00000658-195912000-00012.

19) Brotfain E, Sebbag G, Friger M, et al. Invasive *Candida* infection after upper gastrointestinal tract surgery for

gastric cancer. Int J Surg Oncol. 2017; 2017: 6058567. doi: 10.1155/2017/6058567.

20) Obayashi T, Yoshida M, Mori T, et al. Plasma (1-->3)-beta-D-glucan measurement in diagnosis of invasive deep mycosis and fungal febrile episodes. Lancet. 1995; 345(8941): 17-20. doi: 10.1016/s0140-6736(95)91152-9.

21) Theel ES, Doern CD. β-D-glucan testing is important for diagnosis of invasive fungal infections. J Clin Microbiol. 2013; 51(11): 3478-83. doi: 10.1128/JCM.01737-13.

22) Lamoth F, Akan H, Andes D, et al. Assessment of the role of 1,3-β-d-Glucan testing for the diagnosis of invasive fungal infections in adults. Clin Infect Dis. 2021; 72(Suppl 2): S102-8. doi: 10.1093/cid/ciaa1943.

23) Tonolini M, Bazzi LL, Bianco R. Early non-aneurysmal infectious aortitis: Cross-sectional imaging diagnosis. J Emerg Trauma Shock. 2013; 6(2): 143-5. doi: 10.4103/0974-2700.110811.

24) Nguyen MH, Wissel MC, Shields RK, et al. Performance of *Candida* real-time polymerase chain reaction, β-D-glucan assay, and blood cultures in the diagnosis of invasive candidiasis. Clin Infect Dis. 2012; 54(9): 1240-8. doi: 10.1093/cid/cis200.

25) Clancy CJ, Nguyen MH. Finding the "missing 50%" of invasive candidiasis: how nonculture diagnostics will improve understanding of disease spectrum and transform patient care. Clin Infect Dis. 2013; 56(9): 1284-92. doi: 10.1093/cid/cit006.

26) Karageorgopoulos DE, Vouloumanou EK, Ntziora F, et al. β-D-glucan assay for the diagnosis of invasive fungal infections: a meta-analysis. Clin Infect Dis. 2011; 52(6): 750-70. doi: 10.1093/cid/ciq206.

*Original*

# Isolation frequency of carbapenem-nonsusceptible *Bacteroides* spp. and detection methods for *cfiA*-positive *Bacteroides fragilis*

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**ABSTRACT**

**Aims:** *Bacteroides fragilis* is the most common anaerobic bacteria causing infectious diseases in humans. A *cfiA* gene encoding metallo-β-lactamase, which degrades carbapenem, has been reported as a resistance mechanism against carbapenem. Therefore, the detection of *cfiA* gene is important for determining appropriate antibiotic therapy. Although *cfiA* gene is detected via polymerase chain reaction (PCR), simpler and easier methods are required in clinical settings. We compared the detectability of *cfiA*-positive *B. fragilis* using multiple methods. Moreover, the isolation frequency of carbapenem-nonsusceptible *Bacteroides* spp. was evaluated.
**Methods:** *Bacteroides* spp. was isolated from clinical specimens at the Kanazawa University Hospital. Antimicrobial susceptibility testing was performed via broth microdilution. We explored whether matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) and modified carbapenem inactivation method (mCIM) can show *cfiA* gene positivity.
**Results:** Around 20-50 *Bacteroides* spp. strains/year were isolated from June 2015 to December 2020. Among these, 0-8 strains exhibited meropenem and/or imipenem nonsusceptibility (intermediate and resistant) in each year. An increased rate of carbapenem resistance, including meropenem- and imipenem-nonsusceptible *Bacteroides* spp., was observed. All carbapenem-nonsusceptible *Bacteroides* spp. were susceptible to metronidazole. Four (66.7%) of 6 strains showed *cfiA* gene positivity based on PCR.
**Conclusions:** MALDI-TOF MS and mCIM detected *cfiA*-positive strains. MALDI-TOF MS and mCIM are useful for detecting *cfiA*-positive *B. fragilis*. Because the isolation frequency of carbapenem-nonsusceptible *Bacteroides* spp. is increasing, determining their susceptibility is important for appropriate antimicrobial therapy.
〔Lab Med Int 2025; 4(2): 62-68〕

**Key Words**
*Bacteroides* spp., carbapenem resistance, *cfiA* gene

## I. Introduction

*Bacteroides* spp. are anaerobic bacteria that form normal microbiota in the intestinal tract. They are important pathogenic bacteria for humans in diseased conditions. In particular, antimicrobial-resistant *Bacteroides* spp. infection reportedly shows poor prognosis and prolonged hospitalization[1]. *Bacteroides fragilis* is the most common

anaerobic bacteria causing infectious disease in humans [2]. Carbapenem resistance in *B. fragilis* was first reported in 1983 in Japan[3] and in 1986 in the United States[4]. Although their resistance mechanism to carbapenem remains unclear, the involvement of *cfiA* gene encoding metallo-β-lactamase (MBL), which degradates carbapenem, has been reported [5]. Considering the poor prognosis of carbapenem-resistant *B. fragilis* infection, the detection of *cfiA* gene is important. While *cfiA* gene is detected via polymerase chain reaction (PCR), this method is available only in some hospitals. Therefore, simpler and easier methods are required in clinical settings. In this study, the detectability of *cfiA*-positive *B. fragilis* was compared using multiple methods. Moreover, the isolation frequency of carbapenem-nonsusceptible *Bacteroides* spp., especially *cfiA*-positive strains, was evaluated.

## II. Materials and methods

### *Strains and identification*

*Bacteroides* spp. were isolated from clinical specimens at the Kanazawa University Hospital between June 2015 and December 2020. The specimen was applied and cultured on brucella agar medium (Kyokuto, Tokyo, Japan) for 2 days under anaerobic conditions at 35℃ . The isolated strains were identified using a Bacteroides Bile Esculin agar (BBE Agar, Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan) and AccuDia™ Bacteroides Agar (BA, Shimadzu Diagnostics Corporation, Tokyo, Japan) according to the each directions. *B. fragilis* group were identified by the change of only BBE Agar color from dark brown to black that appears as zones around the colonies. Non-*B. fragilis* group were identified by the growth on BA and no color change in BBE Agar. Those that grew under aerobic conditions were excluded. Eight carbapenem-nonsusceptible strains, which were stored at our hospital, were re-identified using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (MALDI Biotyper, Bruker, Billerica, MA, USA). The strain was applied to the MSP 96 target polished steel BC (Bruker). After drying, 1 μL of 70% formic acid (Beckman Coulter, Brea, CA, USA) was added. Then, the sample was loaded into MALDI Biotyper after 1 μL of HCCA portioned (Bruker) was added. The spectra were analyzed using the MALDI Biotyper Compass software (MBT Compass software) (Bruker) following the manufacturer's instructions.

### *Antimicrobial susceptibility testing*

The strain was suspended in ABCM broth (Eiken Chemical Co., Ltd., Tokyo, Japan) and adjusted with McFarland 2 standards. Then, 25 μL of sample were applied to Brucella Broth Eiken (Eiken Chemical Co., Ltd.). After 100 μL of the bacterial solution was added to Dry Plate Eiken (Eiken Chemical Co., Ltd.), the plate was incubated under anaerobic conditions at 35℃ for 48 h. After 48 h, the growth of the strain was visually observed. The minimum inhibitory concentration (MIC) and interpretive category were determined according to the Clinical and Laboratory Standards Institute M100-ED32 [6]. The additional MIC measurement of metronidazole (MNZ) was performed on 8 carbapenem-nonsusceptible strains, which were stored at our hospital.

### *cfiA gene detection*

*cfiA* gene was detected via PCR. DNA extraction was prepared as follows. The strains were suspended in 200 μL of sterilized water. Then, 5 μL of proteinase K (350 U/mL, Takara Bio Inc., Shiga, Japan) was added, followed by incubation at 50℃ for 5 min. After 7 min of incubation in a boiling water bath, the lysate was centrifuged at 10,000 rpm for 5 min. Then, the supernatant was used as a template. The primers were 5'-CCCAACTCTCGGACAAAGTG-3' and 5'-ACGATCTGCTTGGTATGCTC-3' [7]. PCR was performed using 1 μL of template DNA in a total reaction volume of 25 μL consisting of EmeraldAmp PCR Master Mix (Takara Bio Inc., Shiga, Japan) and 25 pmol of each primer. Reactions were run in a thermal cycler (Biometra, Gottingen, Germany). The PCR program was as follows: 98℃ for 60 s; 30 cycles of 98℃ for 10 s, 56℃ for 30 s, and 72℃ for 60 s; and 72℃ for 60 s. Then, 5 μL of amplicon was analyzed using 2% agarose gel. Strains in which an amplicon was observed at 625 bp were determined to have *cfiA* gene.

### *Detection of cfiA-positive B. fragilis by MALDI-TOF MS*

MALDI Biotyper was used in the same way as for bacterial identification. The MALDI Biotyper Subtyping Module with the MBT Compass software automatically identifies not only bacterial species but also possession of *cfiA* gene. Peaks shifts were reported from 4711 Da and 4817 Da (*cfiA*-negative) to 4688 Da and 4826 Da (*cfiA*-positive), respectively [8]. Strains that matched with the spectra of *cfiA*-positive *B. fragilis* were considered "presumptive *cfiA*-positive".

### *Detection of cfiA-positive B. fragilis using modified carbapenem inactivation method*

We identified carbapenemase-producing *B. fragilis* using modified carbapenem inactivation method (mCIM) [6] with a few changes. Briefly, 1 μL of inoculation loop of

anaerobically cultured strain was firmly suspended in 2 mL of BD BBL™ Trypticase™ Soy Broth (Becton, Dickinson and Company, New Jersey, USA). A meropenem (MEPM) or imipenem (IPM) disk (Eiken Chemical Co., Ltd.), each containing 10 μg of antibiotic, was added and suspended. After incubation under anaerobic conditions at 35℃ for 4 h, the disk was removed from the suspension and placed on a BD BBL™ Muller–Hinton II agar medium (Becton, Dickinson and Company, New Jersey, USA) inoculated with a susceptible *Escherichia coli* indicator (ATCC25922). After incubation under aerobic conditions at 35℃ for 24 h, the diameter of growth inhibition zones was measured. MBL-producing *Klebsiella pneumoniae* was used as a positive control in mCIM analysis. The negative control was a culture medium only.

## III. Results

### *Patient characteristics*

The study period is from June 2015 to December 2020. Carbapenem-nonsusceptible *Bacteroides* spp. were detected in 17 patients. The mean age of patients was 65.0 years, and 10 patients (59.0%) were female. Their underlying diseases were malignant tumors (11 patients, 64.7%), gastrointestinal disorders (6 patients, 35.3%), and hepatobiliary diseases (4 patients, 23.5%). The strains were detected from pus (9 patients, 52.9%), abdominal cavity and subdiaphragmatic drains (4 patients, 23.5%), bile (3 patients, 17.6%), and blood (1 patient, 5.9%). Fifteen specimens (88.2%) were of abdominal origin. Fourteen patients were treated, and 11 patients (78.5%) received antimicrobials. Especially, 7 (63.6%) of

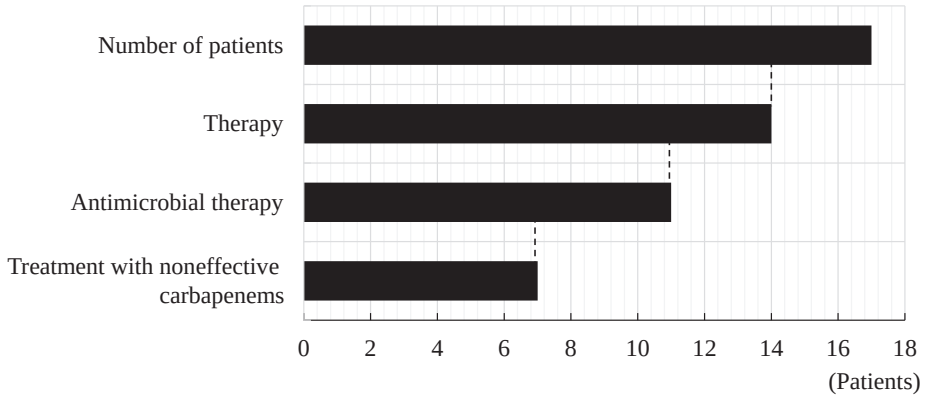
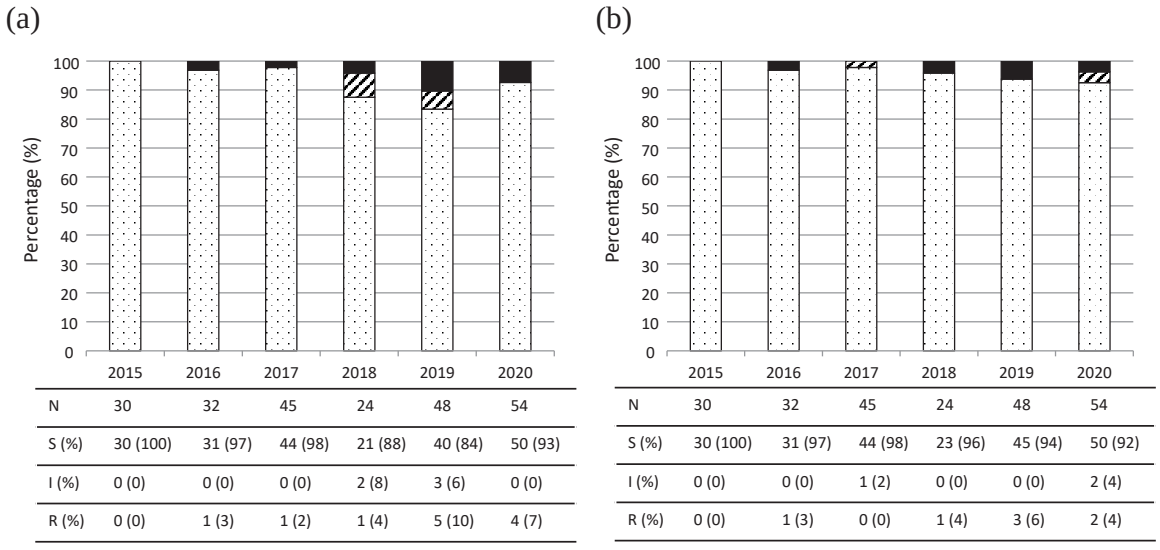



**Figure 1** Treatment of patients infected with carbapenem-nonsusceptible *Bacteroides* spp.
Here, 63.6% of patients were treated with noneffective carbapenems.




| | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 |
|---|---|---|---|---|---|---|
| N | 30 | 32 | 45 | 24 | 48 | 54 |
| S (%) | 30 (100) | 31 (97) | 44 (98) | 21 (88) | 40 (84) | 50 (93) |
| I (%) | 0 (0) | 0 (0) | 0 (0) | 2 (8) | 3 (6) | 0 (0) |
| R (%) | 0 (0) | 1 (3) | 1 (2) | 1 (4) | 5 (10) | 4 (7) |

| | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 |
|---|---|---|---|---|---|---|
| N | 30 | 32 | 45 | 24 | 48 | 54 |
| S (%) | 30 (100) | 31 (97) | 44 (98) | 23 (96) | 45 (94) | 50 (92) |
| I (%) | 0 (0) | 0 (0) | 1 (2) | 0 (0) | 0 (0) | 2 (4) |
| R (%) | 0 (0) | 1 (3) | 0 (0) | 1 (4) | 3 (6) | 2 (4) |

**Figure 2** Changes in carbapenem sensitivity from 2015 to 2020
Sensitivity to meropenem (a) and imipenem (b).
An increased rate of carbapenem-nonsusceptible *Bacteroides* spp. was observed in meropenem and imipenem.
dot: susceptible, oblique line: intermediate, black: resistant, S: susceptible, I: intermediate, R: resistant.

11 patients were treated with noneffective carbapenems (**Fig. 1**).

***Isolation frequency of carbapenem-nonsusceptible Bacteroides spp.***

The characters of isolated 233 *Bacteroides* spp. were described in **Fig. 2**. Two hundred strains were *B. fragilis* group, and 33 strains were non-*B. fragilis* group. Around 20-50 strains/year were isolated in Kanazawa University Hospital. Among these, 0-8 strains exhibited MEPM and/or IMP nonsusceptibility (intermediate and resistant) in each year. Although no MEPM-nonsusceptible *Bacteroides* spp. was detected in 2015, its isolation rate peaked at 16% in 2019 and decreased to 7% in 2020 (**Fig. 2a**). In contrast, the frequency of IPM-nonsusceptible *Bacteroides* spp. increased from 0% in 2015 to 8% in 2020 (**Fig. 2b**). An increased rate of carbapenem resistance, including MEPM- and IPM-nonsusceptible *Bacteroides* spp., was observed during the period.

***Antimicrobial susceptibility of carbapenem-nonsusceptible Bacteroides spp.***

Nineteen strains of carbapenem-nonsusceptible *Bacteroides* spp. were isolated between 2015 and 2020. Seventeen strains were *B. fragilis* group and 2 were non-*B. fragilis* group. Two patients were positive for both *B. fragilis* group and non-*B. fragilis* group. The antimicrobial susceptibility of these strains is shown in **Table 1**. Eight of 19 strains were available for susceptibility tests for MNZ. All of the 8 strains were *B. fragilis* group and showed susceptibility to MNZ.

***Detection of cfiA gene from carbapenem-nonsusceptible B. fragilis***

Six of 8 carbapenem-nonsusceptible strains that were stored at our hospital were re-identified as *B. fragilis* via MALDI-TOF MS. The other 2 strains were re-identified as *B. thetaiotaomicron* and *B. uniformis*, respectively. We investigated whether these 6 *B. fragilis* strains possessed *cfiA* gene by performing PCR. Four strains (66.7%) were *cfiA*-positive. Among the preserved strains, a *cfiA*-positive strain was identified in 2016 and 2020 in each and 2 strains were identified in 2019. Then, MALDI-TOF MS was performed to identify strains with *cfiA* gene (**Table 2**) and was compared to mCIM.

***MALDI-TOF MS detected strains with cfiA gene***

Of 6 *B. fragilis* isolates, 4 strains were detected as presumptive *cfiA*-positive based on MALDI-TOF MS. The other 2 strains were detected as presumptive *cfiA*-negative (**Fig. 3**). The results of *cfiA*-positive *B. fragilis* based on MALDI-TOF MS were consistent with those of *cfiA* gene based on PCR.

***mCIM revealed the susceptibility of strains with/without cfiA gene***

Neither MEPM nor IPM disks inhibited the growth of *E. coli* in mCIM analysis of 4 *cfiA*-positive *B. fragilis*. In the analysis of the other 2 *cfiA*-negative *B. fragilis*, *E. coli* formed growth inhibition zones around MEPM disks, with a diameter larger than 20 mm. IPM disks also inhibited the growth of *E. coli* in the analysis of 2 *cfiA*-negative *B. fragilis*, with growth inhibition zones larger than 18 mm (**Fig. 4**).

**Table 1** Antimicrobial susceptibility of carbapenem-nonsusceptible *Bacteroides* spp.

| | N | S (%) | I (%) | R (%) |
|---|---|---|---|---|
| S/A | 19 | 3 (15.8) | 4 (21.1) | 12 (63.2) |
| T/P | 19 | 9 (47.4) | 1 (5.3) | 9 (47.4) |
| MEPM | 19 | 2 (10.5) | 6 (31.6) | 11 (57.9) |
| IPM | 19 | 9 (47.4) | 3 (15.8) | 7 (36.8) |
| CLDM | 19 | 11 (57.9) | 0 (0.0) | 8 (42.1) |
| MNZ | 8 | 8 (100.0) | 0 (0.0) | 0 (0.0) |

S/A: sulbactam-ampicillin, T/P: tazobactam-piperacillin, MEPM: meropenem, IPM: imipenem, CLDM: clindamycin, MNZ: metronidazole, S: susceptible, I: intermediate, R: resistant.

**Table 2** Detection results of *cfiA*-positive *B. fragilis* via MALDI-TOF MS and mCIM

| cfiA gene by PCR | MALDI-TOF MS | mCIM | | N |
|---|---|---|---|---|
| | | MEPM (mm) | IPM (mm) | |
| Detected | Detected | 0 | 0 | 4 |
| Not detected | Not detected | ≥ 20 | ≥ 18 | 2 |

PCR: polymerase chain reaction, MALDI-TOF MS: matrix-assisted laser desorption ionization–time of flight mass spectrometry, mCIM: modified carbapenem inactivation method, MEPM: meropenem, IPM: imipenem.

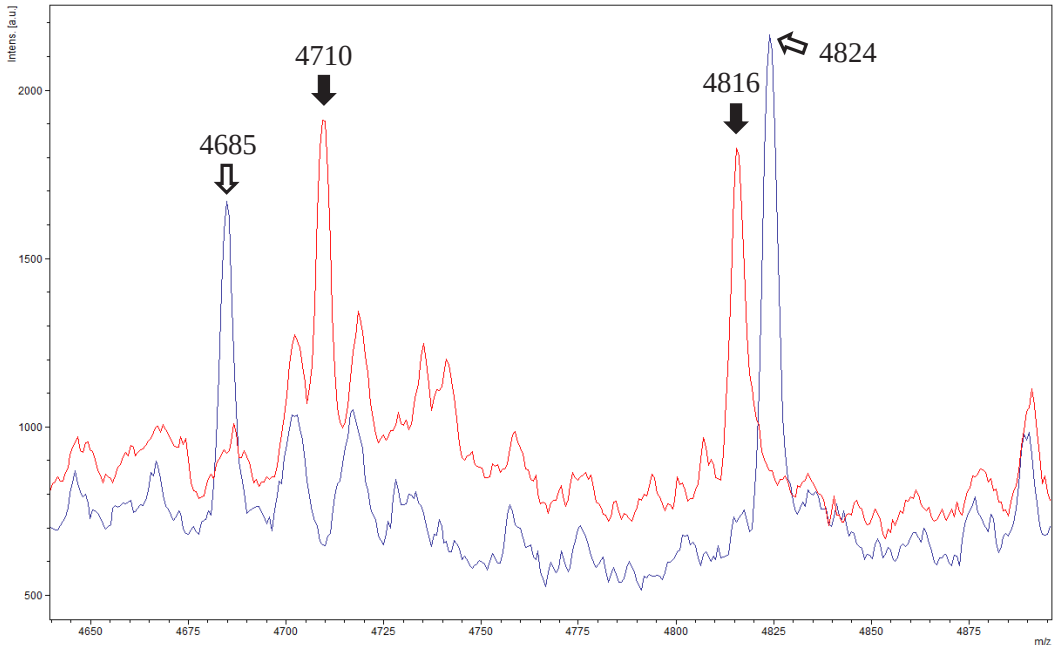



**Figure 3** MALDI-TOF MS peaks of *cfiA*-negative and *cfiA*-positive *B. fragilis*.
White arrows indicate peaks of *cfiA*-positive *B. fragilis*. Black arrows indicate peaks of *cfiA*-negative *B. fragilis*.

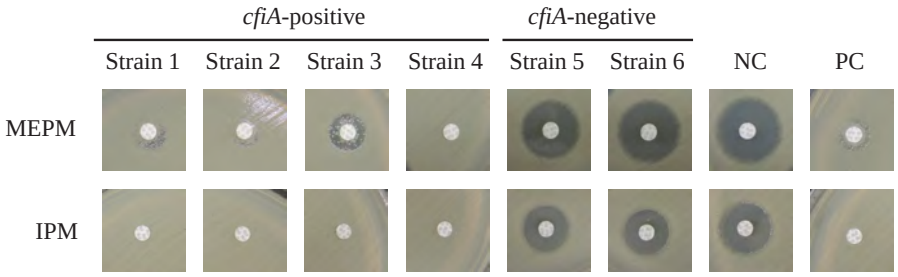



**Figure 4** Results of modified carbapenem inactivation method
*B. fragilis* strains 1, 2, 3, 4 were *cfiA*-positive, whereas strains 5 and 6 were *cfiA*-negative. NC is the result of a bacteria-free test. PC is the result of metallo-β-lactamase-producing *Klebsiella pneumoniae*.
MEPM: meropenem, IPM: imipenem, NC: negative control, PC: positive control.

## IV. Discussion

The isolation frequency of carbapenem-nonsusceptible *Bacteroides* spp. was investigated, and methods for detecting *cfiA*-positive *B. fragilis* were examined. This study revealed that the maximum isolation frequency of MEPM- and IPM-nonsusceptible *Bacteroides* spp. was 16% and 8%, respectively. The isolation rate of carbapenem-nonsusceptible *Bacteroides* spp. increased over the past 6 years. Recent reports indicate that carbapenem-nonsusceptible *Bacteroides* spp. have been isolated in some countries with various frequencies[9)-11)]. The increased frequency of carbapenem-resistant *Bacteroides* spp. was also reported in some studies[9),12),13)]. The isolation frequency increased similar to that reported in other studies in Japan[9)]. Therefore, monitoring the isolation frequency of carbapenem-nonsusceptible *Bacteroides* spp. is important in hospitals. Moreover, the detection of carbapenem-nonsusceptible *Bacteroides* spp. is important for determining appropriate antibiotic therapy. We should have investigated the isolation frequency of carbapenem-nonsusceptible *B. fragilis*. Although MALDI-TOF MS analysis identify strains to *B. fragilis*, the equipment was not available during the study period in our facility. Therefore, we investigated the isolation frequency of carbapenem-nonsusceptible strains in the genus *Bacteroides*.

The isolation rate of MEPM-nonsusceptible *Bacteroides* spp. peaked at 16% in 2019 and decreased to 7% in 2020. Although the reason for the reduced MEPM-nonsusceptible *Bacteroides* spp. frequency is not clear, small sample number may affect the change of frequency in this case.

In our study, while 4 strains (66.7%) of carbapenem-nonsusceptible *B. fragilis* were *cfiA*-positive, the other 2 strains did not possess the gene. Other than *cfiA* gene, the mechanisms of carbapenem nonsusceptibility remain unclear[14]. One study showed that functional changes in efflux pumps affect carbapenem sensitivity[15]. Further studies are needed to clarify the resistance mechanisms, especially in 2 carbapenem-nonsusceptible *B. fragilis* without *cfiA* gene.

To date, there is no routine method to detect *cfiA*-positive *B. fragilis*. In this study, MALDI-TOF MS and mCIM were useful in detecting *cfiA*-positive *B. fragilis*. A recent study reported that the sensitivity and specificity of MALDI-TOF MS in detecting *cfiA*-positive *B. fragilis* were 100.0% and 99.7%, respectively[16]. Although MALDI-TOF MS is a simple and quick method, it can be performed only in the facilities with the required equipment. In facilities where MALDI-TOF MS is unavailable, mCIM may be useful. Because mCIM does not require specialized equipment or reagents, it is widely used and can be performed at any facility. Therefore, MALDI-TOF MS and mCIM might provide earlier and more accurate results in determining *cfiA*-positive strains. This will help determine appropriate antimicrobial use for treating carbapenem-nonsusceptible *B. fragilis* infection.

In summary, even if MALDI-TOF MS or PCR is not available, mCIM is useful for detecting *cfiA*-positive *B. fragilis*. Because the isolation frequency of carbapenem-nonsusceptible *Bacteroides* spp. is increasing, determining its susceptibility is important for appropriate antimicrobial therapy.

**Declarations**

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**Conflicts of interest:**

The authors have declared that no conflict of interest exists.

**Ethics approval:**

All experiments were performed in accordance with approved guidelines of Kanazawa University. This study was conducted with the approval of the ethics committee of Kanazawa University (approval number: 3596).

**Authorship contributions:**

YTS and YI designed the study and interpreted the data. YTS performed experiment and wrote the draft of the manuscript together with YI. All authors read the manuscript and commented.

**References**

1) Hartmeyer GN, Sóki J, Nagy E, et al. Multidrug-resistant *Bacteroides fragilis* group on the rise in Europe? J Med Microbiol. 2012; 61 (Pt 12) : 1784-8.

2) Jasemi S, Emaneini M, Ahmadinejad Z, et al. Antibiotic resistance pattern of *Bacteroides fragilis* isolated from clinical and colorectal specimens. Ann Clin Microbiol Antimicrob. 2021; 20 (1) : 27-34.

3) Yotsuji A, Minami S, Inoue M, et al. Properties of Novel 3-Lactamase Produced by *Bacteroides fragilis*. Antimicrob Agents Chemother. 1983; 24 (6) : 925-9.

4) Cuchural GJJr, Malamy MH, Tally FP. Beta-Lactamase-Mediated Imipenem Resistance in *Bacteroides fragilis*. Antimicrob Agents Chemother. 1986; 30 (5) : 645-8.

5) Thompson JS, Malamy MH. Sequencing the Gene for an Imipenem-Cefoxitin-Hydrolyzing Enzyme (CfiA) from *Bacteroides fragilis* TAL2480 Reveals Strong Similarity between CfiA and Bacillus cereus, B-Lactamase II. J Bacteriol. 1990; 172 (5) : 2584-93.

6) CLSI M100 ED32:2022. http://em100.edaptivedocs.net/

7) Yamazoe K, Kato N, Kato H, et al. Distribution of the *cfiA* Gene among *Bacteroides fragilis* Strains in Japan and Relatedness of *cfiA* to Imipenem Resistance. Antimicrob Agents Chemother. 1999; 43 (11) : 2808-10.

8) Nagy E, Becker S, Sóki J, et al. Differentiation of division I (*cfiA*-negative) and division II (*cfiA*-positive) *Bacteroides fragilis* strains by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. J Med Microbiol. 2011; 60 (Pt 11) : 1584-90.

9) Ueda T, Takesue Y, Matsumoto T, et al. Change in antimicrobial susceptibility of pathogens isolated from surgical site infections over the past decade in Japanese nation-wide surveillance study. J Infect Chemother. 2021; 27 (7) : 931-9.

10) Wang Y, Han Y, Shen H, et al. Higher Prevalence of Multi-Antimicrobial Resistant *Bacteroides spp*. Strains Isolated at a Tertiary Teaching Hospital in China. Infect Drug Resist. 2020; 13: 1537-46.

11) König E, Ziegler HP, Tribus J, et al. Surveillance of Antimicrobial Susceptibility of Anaerobe Clinical Isolates in Southeast Austria: *Bacteroides fragilis* Group Is on the Fast Track to Resistance. Antibiotic. 2021; 10 (5) : 479-85.

12) Copsey-Mawer S, Hughes H, Scotford S, et al. UK *Bacteroides* species surveillance survey: Change in antimicrobial resistance over 16 years (2000-2016). Anaerobe. 2021; 72: 102447.

13) Liu CY, Huang YT, Liao CH, et al. Increasing Trends in Anti-

microbial Resistance among Clinically Important Anaerobes and Bacteroides fragilis Isolates Causing Nosocomial Infections: Emerging Resistance to Carbapenems. Antimicrob Agents Chemother. 2008; 52(9): 3161-8.

14) Wexler HM. *Bacteroides*: the Good, the Bad, and the Nitty-Gritty. Clin Microbiol Rev. 2007; 20(4): 593-621.

15) Pumbwe L, Chang A, Smith RL, et al. Clinical significance of overexpression of multiple RND-family efflux pumps in *Bacteroides fragilis* isolates. J Antimicrob Chemother. 2006; 58(3): 543-8.

16) Kawamoto Y, Kosai K, Ota K, et al. Rapid detection and surveillance of cfiA-positive *Bacteroides fragilis* using matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Anaerobe. 2021; 72: 102448.

*Original*

# Morphological and Genetic Analysis of *Diploscapter coronatus*: Insights into Identification Challenges and Species Relationships

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**ABSTRACT**

**Objective:** This study aims to address the challenges of identifying *Diploscapter coronatus* by morphological and genetic analysis. The nematode was previously identified morphologically in fecal samples from a patient with Henoch-Schönlein purpura. We used genetic methods to complement and refine species identification.
**Materials and Methods:** Nematodes isolated from the patient's feces were fixed in formalin, and morphological observations were made under a light microscope. PCR amplification of the SSU rRNA and Hsp90 genes was performed on DNA extracted from the nematodes during the same period. The resulting DNA was cloned, sequenced, and subjected to phylogenetic analysis using the neighbor-joining method.
**Results:** Morphological observations confirmed that the nematode shared key features with *D. coronatus*. The SSU rRNA analysis showed 99% similarity with 11 *Diploscapter* species, including *D. coronatus*. However, Hsp90 gene analysis placed the nematode in the *D. lycostoma* cluster, revealing inconsistencies between morphological and genetic data.
**Conclusion:** The present study highlights the complexity of species identification within the genus *Diploscapter* that results from the limited genetic information and overlapping morphological characters. The results underscore the need for more extensive genetic data and comprehensive phylogenetic analyses to resolve these identification challenges and better understand species relationships within the genus.
〔Lab Med Int 2025; 4(2): 69-74〕

**Key Words**
*Diploscapter coronatus*, *Diploscapter* spp., genetic analysis, small subunit ribosomal RNA (SSU rRNA), heat shock protein 90 (Hsp90)

## I. Introduction

*Diploscapter coronatus* was first described by Cobb in 1893 as a free-living nematode typically found in soil surrounding decaying plants and roots[1]. Its detection in human samples is very rare, although its presence has been noted in past cases of anoxia and alkaline urine[2),3)]. In addition, recent reports have identified nematodes suspected of being this species in clinical samples such as urine and feces[4),5)]. Several species, including *D. coronatus*, have been documented within the genus *Diploscapter*. In 2002, we detected this nematode in fecal samples from a patient with Henoch-Schönlein purpura, identified by its morphological features[6)]. Recently, both morphological and genetic methods have been used for species identification in nematode studies[7)]. In this study, we report the analysis of a sample of *D. coronatus*, previously identified by morphological observations and now complemented by genetic identification methods.

## II. Materials and Methods

### 1. Samples

Nematodes found in patient feces in 2002 were cultured in nematode growth medium (NGM), then fixed in 10% buffered formalin and stored at room temperature. Morphological observations were carried out on these formalin-fixed nematodes. During the same period, genomic DNA was extracted from *D. coronatus* nematodes derived from the patient and cultured in NGM using a QIAamp DNA Micro Kit (Qiagen, Hilden, Germany) and stored frozen at −80℃. This study was approved by the Ethics Committee of Kochi University Medical School, Japan (No.30-87).

### 2. Morphology

Morphological observations and measurements of the formalin-fixed nematodes were made using a light microscope and considered in conjunction with the observations we have previously reported[6]. Using De Man's formula[8], specific ratios and indices were calculated based on measurements of different parts of the nematode's body to describe its morphological characteristics.

### 3. Polymerase Chain Reaction (PCR) and Cloning

The primers used for the small subunit ribosomal RNA (SSU rRNA) gene were obtained from Holterman et al[9]. (2006), and those for the 90 kDa heat shock protein (Hsp90) gene were obtained from Zeng Qi Zhao[7] (2013), respectively. The 20 μL PCR reactions contained 10 μL KAPA2G Fast PCR Kit (Kapa Biosystems, USA), 1 μL (0.05 μM) of each forward and reverse primer, and 2 μL of DNA template. The thermal cycling program was as follows: denaturation at 95℃ for 3 min, followed by 30 cycles of denaturation at 94℃ for 30 s, annealing at 55℃ for 30 s, and extension at 72℃ for 45 s. A final extension was performed at 72℃ for 10 min. The amplified DNA was purified using a QIAGEN Gel Extraction Kit (QIAGEN, Valencia, CA, USA). The cDNA was ligated into a T-vector using the DynaExpress TA PCR Cloning Kit (BioDynamics Laboratory, Tokyo, Japan) and then transformed into competent *Escherichia coli* DH5α cells (FUJIFILM Wako, Tokyo, Japan) using the heat shock method. Finally, the recombinant plasmid was extracted from the transformed *E. coli*, using EZ-10 Spin Column Plasmid DNA Miniprep Kit (BIO BASIC, Ontario, Canada).

### 4. Sequencing and Phylogenetic Analysis

Purified plasmids were sequenced using the Big Dye™ Terminator Cycle Sequencing Ready Reaction Mix v3.1 Kit. Cycle sequencing products were purified using the FastGene Dye Terminator Removal Kit (NIPPON Genetics, Tokyo, Japan) and analyzed on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The genetic sequences obtained were subjected

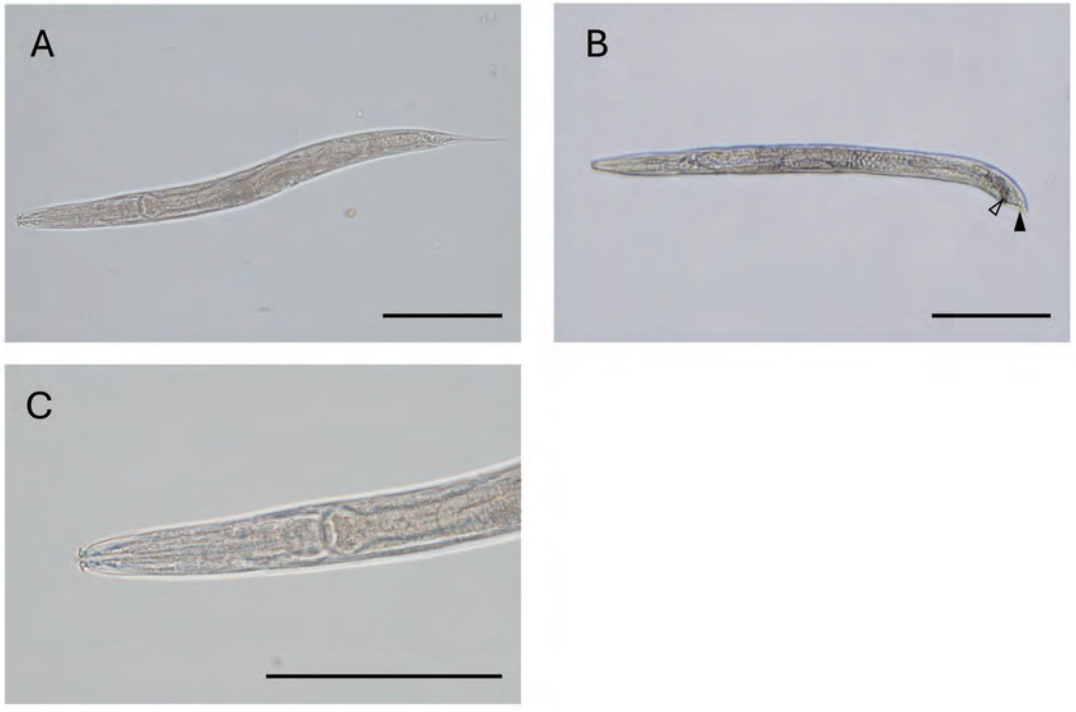


**Figure1** Microscopic morphology of adult nematodes.
Whole body of both adult female (A) and male exhibiting the presence of spicules (△) and caudal alae (▲) in the tail (B). Enlarged view of the head of an adult female (C). Scale bar indicating 100 μm.

to similarity searches using BLAST[10] from the National Center for Biotechnology Information (NCBI). DNA sequences were aligned using Clustal OMEGA[11] and a phylogenetic tree was constructed using the Neighbor-Joining method with MEGA 11[12]. The root of the phylogenetic tree was constructed using the nucleotide sequence of *Rhabditis myriophila* (U81588) for SSU, and *Acrobeloides amurensis* (DQ340377) and *Cephalobus cubaensis* (DQ340378) for HSP90.

## III. Results

### 1. Morphological Observations

The microscopic images of the male and female nematodes in this study are shown in **Figure 1**. The length of the adult worms ranged from 344 to 522 μm, with a width of 22 to 39 μm (**Table 1**). They had two characteristic pairs of lips on the head, with the dorsal and ventral lips being hook-shaped and the second pair branching anteriorly, consistent with the characteristics of the genus *Diploscapter* (**Figure 1B**). To compare this nematode with the genus *Diploscapter*, coefficients calculated from the De Man measurements are presented in **Table 1**. Morphologically, both the size and the De Man ratios were similar to those of *D. coronatus*.

### 2. Genetic Findings

A homology search using BLAST showed that the SSU rRNA region of this nematode exhibited 99% similarity to 11 *Diploscapter* species. The similarity to the two included *D. coronatus* sequences was 99.3%. In the Hsp90 region, similarity ranged from 89.8% to 99.4% for *D. lycostoma* and from 86.8% to 89.7% for *D. coronatus* (**Table 2**). Phylogenetic analysis classified the *Diploscapter* species into clusters based on the four SSU regions, with the nematode in this study placed in a cluster that includ-

**Table 1** Comparative measurements of adluts *Diploscapter* spp.*

| Author(s) | *D. coronatus* | | | | | *D. lycostoma* |
|---|---|---|---|---|---|---|
| | This case | Morimoto et al (2006) | Loof (1964) | Abebe (1998) | Abolafia&Pena-Santiago (2007) | Wahab (1962) |
| Length body** | 344 - 522 | 348 - 394 | 250 - 400 | 350 - 427 | 358 - 504 | 519 - 546 |
| a | 13.4 - 17.3 | | 14 - 18 | 16 - 20 | 15 - 18 | 15.2 - 16.7 |
| b | 4.4 - 5.6 | | 3.5 - 4.5 | 3.6 - 4.4 | 4.0 - 5.3 | 6.3 - 6.5 |
| c | 5.3 - 8.4 | | 5.9 - 8.2 | 5.8 - 9.6 | 5.7 - 7.4 | 9.4 - 12.1 |
| c' | 4.0 - 7.1 | | | 3.5 - 6.5 | 4.4 - 6.8 | |
| V | 45 - 53 | | 51 - 57 | 50 - 58 | 48 - 57 | 56 |

*The De Man ratios (a to c'+V) Favoured by plant and soil nematologists are as follows: a=body length/maximun width; b=body length/length of esophagus; c=body length/tail length; V= Percentage of body length from the head to the vulva.
**Length body (μm).

**Table 2** Homology search in the SSU rRNA region using BLAST

| Nematodes | Assession # | Max Score | Total Score | Sequence Coverage (%) | E value | Sequence identity (%) |
|---|---|---|---|---|---|---|
| *Diploscapter* sp. | EU196003.1 | 1572 | 1572 | 100% | 0.0 | 99.65% |
| *Diploscapter* sp. | U81586.1 | 1572 | 1572 | 100% | 0.0 | 99.65% |
| *Diploscapter* sp. | AF083009.1 | 1565 | 1565 | 100% | 0.0 | 99.42% |
| *Diploscapter coronatus* | KJ636377.1 | 1557 | 1557 | 100% | 0.0 | 99.30% |
| *Diploscapter coronatus* | AY593921.1 | 1555 | 1555 | 100% | 0.0 | 99.30% |
| *Diploscapter* sp. | OP964516.1 | 1550 | 1550 | 100% | 0.0 | 99.19% |
| *Diploscapter* sp. | OP964515.1 | 1550 | 1550 | 100% | 0.0 | 99.19% |
| *Diploscapter* sp. | OP964514.1 | 1550 | 1550 | 100% | 0.0 | 99.19% |
| *Diploscapter formicidae* | JQ838254.1 | 1550 | 1550 | 100% | 0.0 | 99.19% |
| *Protorhabditis* sp. | EU196002.1 | 1428 | 1428 | 100% | 0.0 | 96.64% |
| *Protorhabditis* sp. | AF083024.1 | 1356 | 1356 | 100% | 0.0 | 95.13% |
| *Heterorhabditis indica* | LN611143.1 | 1166 | 1166 | 100% | 0.0 | 91.35% |

Homology search using BLAST was performed with the nucleotide sequences obtained from sequencing the plasmid DNA, which contained the PCR product of the SSU region of this nematode.

ed *D. coronatus* (**Figure 2**). In contrast, using the Hsp90 data, the *Diploscapter* species were classified into clusters corresponding to *D. coronatus*, *D. formicidae* and *D. lycostoma* with the nematode in this study placed in the *D. lycostoma* cluster (**Figure 3**).

## IV. Discussion

Morphologically, it has been estimated that there are approximately 15 species within the genus *Diploscapter*[13]. However, to date, comprehensive genetic analyses and electron microscopic evaluations have not been carried out for most species. The nematode analyzed in this study matched the morphological characteristics of *D. coronatus* observed in previous studies[14)-17)]. In addition, the b-ratio (body length/esophagus length) of *D. coronatus* differed from that of *D. lycostoma* in the De Man ratios referenced for nematodes[17]. From these findings, the nematode in this case demonstrated a b-ratio of 4.4-5.6,

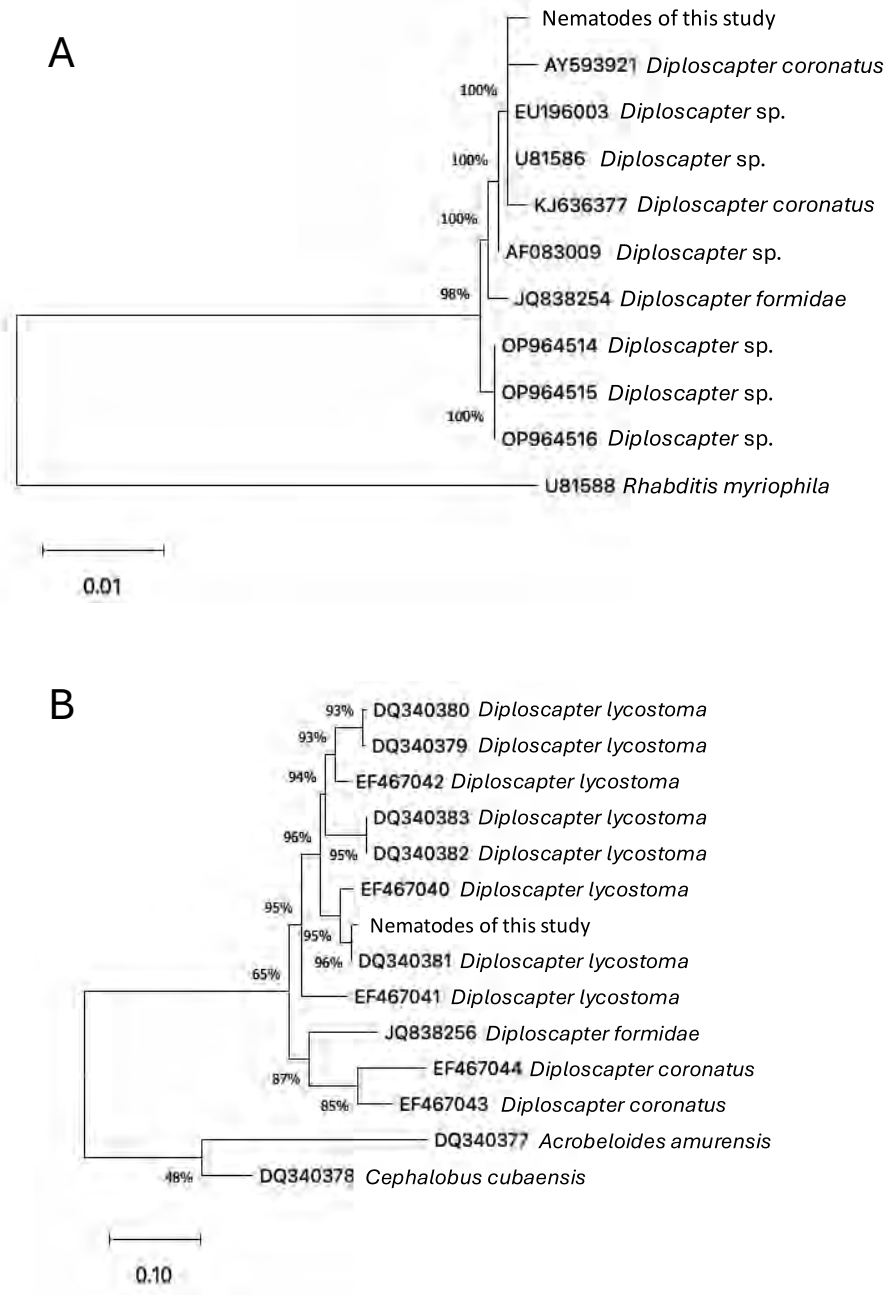


**Figure 2** Neighbor-joining phylogenetic trees inferred from SSU rRNA gene (A) and HSP90 gene (B).
The phylogenetic trees include sequences from 9 species for SSU rRNA DNA and from 11 species for the HSP90 gene within the genus *Diploscapter*, along with sequences from other related taxa. Bootstrap values are shown at the nodes. The scale bar indicates the number of nucleotide substitutions per site. Sequences from *Rhabditis myriophila* and other outgroup species were used to root the tree.

**Table 3** Homology search in the HSP90 region using BLAST

| Nematodes | Assession # | Max Score | Total Score | Sequence Coverage (%) | E value | Sequence identity (%) |
|---|---|---|---|---|---|---|
| *Diploscapter lycostoma* | DQ340381 | 1286 | 1286 | 99% | 0.0 | 99.44% |
| *Diploscapter lycostoma* | EF467040 | 1168 | 1168 | 100% | 0.0 | 96.48% |
| *Diploscapter lycostoma* | EF467042 | 1088 | 1088 | 100% | 0.0 | 94.52% |
| *Diploscapter lycostoma* | DQ340382 | 1088 | 1088 | 99% | 0.0 | 94.63% |
| *Diploscapter lycostoma* | EF467041 | 1050 | 1050 | 100% | 0.0 | 93.58% |
| *Diploscapter lycostoma* | DQ340379 | 907 | 907 | 99% | 0.0 | 90.31% |
| *Diploscapter formicidae* | JQ838256 | 885 | 885 | 96% | 0.0 | 90.39% |
| *Diploscapter lycostoma* | DQ340380 | 881 | 881 | 99% | 0.0 | 89.75% |
| *Diploscapter coronatus* | EF467044 | 765 | 765 | 100% | 0.0 | 86.76% |
| *Diploscapter coronatus* | EF467043 | 702 | 702 | 79% | 0.0 | 89.72% |
| *Leguminivora glycinivorella* | MK343468 | 174 | 174 | 20% | 1e-38 | 88.28% |

Homology search using BLAST was performed with the nucleotide sequences obtained from sequencing the plasmid DNA, which contained the PCR product of the HSP90 region of this nematode.

which morphologically identified it as *D. coronatus*.

The information available in NCBI on the genus *Diploscapter* is particularly limited, and only three species are fully documented: *D. coronatus*, *D. lycostoma* and the newly described *D. formicidae*. The nematode in this study showed high similarity to *D. coronatus* in the SSU rRNA gene, while in the Hsp90 region, it exhibited higher similarity to *D. lycostoma*. We currently lack sufficient genetic data on the SSU rRNA region in *D. lycostoma*. In addition, the genetic information available for the Hsp90 region lacks corresponding, detailed morphological descriptions. These factors likely contribute to the inconsistencies observed using genetic identification methods. In practice, when performing a BLAST search for the Hsp90 gene sequence of *D. lycostoma* (EF467042), the similarity to other *D. lycostoma* sequences ranges from 91.4% to 93.6%, and to *D. coronatus* sequences ranges from 85.8% to 85.9%. Identification by genetic methods is therefore challenging.

Interestingly, G. Markin *et al*[18] reported that *D. coronatus* (Cobb 1893[1]) is synonymous with *D. lycostoma* (Volk 1950)[19]. The paucity of samples available for phylogenetic analysis may suggest that the resulting phylogenetic tree does not accurately reflect the true evolutionary relationships between the classified groups.

To date, while *D. coronatus* has been detected in human samples[4, 5], there have been no reports of other species within the genus *Diploscapter* being detected in humans.

In addition, the detection of *Diploscapter formicidae*[7] and *Diploscapter lycostoma*[19, 20] in association with ants suggests a possible correlation between species and host specificity. In general, when a rare parasite is detected in humans, species identification is often performed based on the similarity of gene sequences from a particular genetic region, and genetic identification for various organisms has advanced in clinical examination. However, for the genus *Diploscapter*, the current genetic information available is insufficient, leading to uncertainties in species identification. In fact, Arai et al[21] reported that although they attempted to genetically identify *Diploscapter* from human samples, a definitive identification of *D. coronatus* could not be achieved because of limited nucleotide sequence information.

Interestingly, although males are thought to be absent in the genus *Diploscapter*, we have reported the presence of males in this genus[6]. Furthermore, the presence of males in *D. coronatus* has also been reported by other researchers[4, 22]. If males could be found in other species of the genus *Diploscapter*, it might be possible to identify species based on characteristics such as male reproductive organs.

## V. Conclusion

The identification of species within the genus *Diploscapter* requires more extensive morphological and molecular data, with particular emphasis on comparing genetic information among different species to advance phylogenetic analyses. In addition, the ability to specifically detect species will likely provide important insights into host specificity.

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### Authorship contributions

All authors were involved in the preparation of the manuscript and reviewed the final manuscript.

## Disclosure of Conflicts of Interest

We declare that we have no conflicts of interest.

### References


1) Cobb NA. 1893. Nematodes, mostly Australian and Fijian. Department of Agriculture, New South Wales. Miscellaneous Publication No. 13: 3-59.
2) Chandler AC. Diploscapter coronata as a facultative parasite of Man, with a general review of vertebrate parasitism by rhabditoid worms. Parasitology. 1938; 30 (1): 44-55.
3) Yokogawa S. A human case of accidental parasitism of Diploscapter coronata (Cobb, 1893) Cobb, 1913. Zool Mag. 1936; 48: 507-11. (Japanese with English summary)
4) Watthanakulpanich D, Anantaphruti MT, Maipanich W. Diploscapter coronata infection in Thailand: report of the first case. Southeast Asian J Trop Med Public Health. 2005; 36 (Suppl 4): 99-101.
5) Athari A, Mahmoudi MR. Diploscapter coronata Infection in Iran: Report of the First Case and Review of Literature. Iranian J Parasitol. 2008; 3 (3): 42-7.
6) Morimoto N, Korenaga M, Yagyu K, et al. Morphological observations and the effects of artificial digestive fluids on the survival of Diploscapter coronata from a Japanese patient. J. Helminthol. 2006; 80 (4): 341-48.
7) Zhao ZQ, Davies KA, Brenton-Rule EC, et al. Diploscapter formicidae sp. n. (Rhabditida: Diploscapteridae), from the ant Prolasius advenus (Hymenoptera: Formicidae) in New Zealand. Nematology. 2013; 15 (1): 109-23. doi:10.1163/156854112X649828.
8) De Man JG. On the free-living nematoids and some other animals of brackish water and fresh water. Tijdschrift der Nederlandsche Dierkundige Vereeniging. 1880; 5: 1-103.
9) Holterman M, van der Wurff A, van den Elsen S, et al. Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. Mol Bio Evol. 2006; 23 (9) :1792-800. doi.org/10.1093/molbev/msl044.
10) Altschul SF, Madden TL, Schäffer AA, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 1997; 25 (17): 3389-402. doi: 10.1093/nar/25.17.3389.
11) Sievers F, Wilm A, Dineen D, et al. Fast, scalable generation of high−quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol. 2011; 7: 539. doi: 10.1038/msb. 2011.75
12) Tamura K, Stecher G, Kumar S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Mol Biol Evol. 2021; 38 (7): 3022-27. doi: 10.1093/molbev/msab120.
13) Sudhaus W. Phylogenetic systematisation and catalogue of paraphyletic "Rhabditidae" (Secernentea, Nematoda). J Nematode Morphol Syst. 2011; 14 (2): 113-78.
14) Loof PAA. Free-living and plant-parasitic nematodes from Venezuela. Nematologica. 1964; 10: 201-300.
15) Abebe E, Karegar A, Nabil H, et al. A redescription and ultrastructural study of Diploscapter coronatus (Cobb, 1983) Cobb, 1913 from Ethiopia and Iran. Russ. J Nematol. 1998; 6 (1): 17-22.
16) Abolafia J, Peña-Santiago R. Nematodes of the order Rhabditida from Andalucía Oriental, Spain. The genera Protorhabditis (Osche, 1952) Dougherty, 1953 and Diploscapter Cobb, 1913, with description of P. spiculocrestata sp. n. and a species Protorhabditis key. J Nematol. 2007; 39 (3): 263-74.
17) Wahab A. Untersuchungen über Nematoden in den Drüsen des Kopfes der Ameisen (Formicidae). Zeitschrift für Morphologie und Ökologie der Tiere. 1962; 52 (1): 33-92.
18) Markin GP, McCoy CW. The Occurrence of a Nemotode, *Diploscapter lycostoma*, in the Pharyngeal Glands of the Argentine Ant, *Iridomyrmex humilis*. Ann Entomol Soc Am. 1968; 61 (2): 505-9. doi.org/10.1093/aesa/61.2.505
19) Völk J. Die Nematoden der Regenwürmer und aasbesuchenden Käfer. Zoologische Jahrbücher (Systematik) 1950; 79: 1-70. [Cited in: Tahseen Q, Siddiqi M.R, Rowe J. Study on species of Diploscapter Cobb (Nematoda: Rhabditida) from India including D. indicus sp. n. Int J Nematol. 2002; 12: 183-88.
20) Lynn C, Andrea S, Eric E. Low temperature SEM and molecular phylogenetics of three Diploscapter populations. J Nematol 2006; 38 (2): 36.
21) Arai M, Nishimura K, Miyauchi H, et al. A large number of nematodes found in the urinary sediment of a 79 years-old man. Clin Parasitol. 2015; 26 (1): 39-42. (in Japanese)
22) Shah AA, Vaid S. Description of Sclerorhabditis miniata n. sp. and First Description of Male of Diploscapter coronatus Cobb, 1913 (Nematoda: Rhabditidae). J Nematol. 2015; 47 (2): 153-8.