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A case of cervical subcutaneous abscess due to *Bordetella hinzii*

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ABSTRACT

We present a case of subcutaneous infection caused by *Bordetella hinzii* in a healthy male. The isolate was successfully identified by *gyrB* gene sequencing. *B. hinzii* cannot be distinctively identified using 16S rRNA gene sequencing or by biochemical methods. The number of cases infected with *B. hinzii* might be underestimated owing to the difficulty in accurate identification, which can be achieved by *gyrB* gene sequencing to gain knowledge about the species.

1. Introduction

Bordetella hinzii is a strictly aerobic and glucose non-fermenting gram-negative rod, which was firstly reported in 1995 (Vandamme et al., 1995). This species was isolated from poultry, causing respiratory infections in them, and from rodents (Hayashimoto et al., 2008; Jiyipong et al., 2013; Register & Kunkle, 2009; Vandamme et al., 1995). *B. hinzii* rarely infects humans, mostly immunocompromised, and a few cases including pulmonary infection (Fabre et al., 2015); Funke et al.,1996; (Palacián Ruiz et al., 2013), cholangitis (Arvand et al., 2004), endocarditis (González et al., 2019), and bacteremia (Cookson et al., 1994; Fry et al., 2007; Hristov et al., 2008; Kattar et al., 2000) have been reported. Here we report a case of *B. hinzii* infection that was identified using *gyrB* gene sequencing technique. To the best of our knowledge, this is the first report of the case with a subcutaneous abscess due to *B. hinzii*, in an immunocompetent individual without underlying disease.

1.1. Case report

A 63-year-old healthy male without underlying disease, complained of a subcutaneous abscess on his neck, which developed rapidly and was tender. He was a farmer who cultivated water melons and Chinese yam. He was administered cefditoren pivoxil, amoxicillin-clavulanate, and garenoxacin by the previous doctors; however, there was no improvement. Sixteen days after the onset, he was referred to the Shinshu University Hospital. At the first visit, the abscess was approximately five centimeters in diameter on palpation, and laboratory data showed a C-reactive protein concentration of 0.76 mg/dL, indicating mild inflammation, and a leukocyte count of 6620/μL. The first sampling of abscess fluid was conducted and cefditoren pivoxil (100 mg three times daily) was empirically administrated for 4 days as it is a broad-spectrum oral antimicrobial. He visited the outpatient department after 2 weeks but showed no improvement. A second sampling of the abscess fluid was conducted, and trimethoprim-sulfamethoxazole (800 mg of sulfamethoxazole and 160 mg of trimethoprim twice daily) was administrated for 2 weeks, based on the result of the antimicrobial susceptibility testing. However, 2 weeks later, no apparent improvement was observed during the outpatient visit. Therefore, a third sampling of the abscess fluid was carried out and, taking into consideration the necrotizing lymphadenitis, prednisolone (5 mg three times daily) was administrated for 2 weeks instead of antimicrobials. However, no apparent improvement was observed, thus, antimicrobial therapy with sitafloxacin (100 mg once daily), which showed low MIC in the antimicrobial susceptibility testing, was resumed 1 week after initiating prednisolone. Reduction in size of the abscess was observed 23 days after initiating sitafloxacin treatment. Sitafloxacin was discontinued 78 days later. The last sampling of the abscess fluid, in which purulent fluid was not drained, was taken the day before sitafloxacin was discontinued. The abscess became impalpable within 1 year. No deterioration has been observed for 3 years. Pathologically, malignant cells were not observed in the abscess fluid.

Except for the last sampling, whose culture was negative, a small number of tiny gram-negative rods were observed in the first three

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samples of abscess fluid. No bacteria were observed when the direct smears were Gram stained. The cultures were performed using TSAII with 5% Sheep Blood / Drigalski Agar, Modified (Nippon Becton Dickinson, Tokyo, Japan) in aerobic conditions at 35 °C for 2 days; Chocolate II Agar (Nippon Becton Dickinson, Tokyo, Japan) in 5% CO_2 at 35 °C for 2 days; Anaero Columbia Agar with rabbit blood (Nippon Becton Dickinson, Tokyo, Japan) in anaerobic conditions at 37 °C for 3 days; and HK semi-fluid medium (Kyokuto Pharmaceutical Industrial, Tokyo Japan) in aerobic conditions at 35 °C for a week. All samples of the abscess fluid were collected aseptically by fine-needle aspiration, and growth of other bacteria, including skin flora, was not observed. Acid-fast bacteria culture was performed for the first two samples and both cultures were negative after 8 weeks. The rods formed smooth, round, whitish, and non-hemolytic colonies on 5% sheep blood agar plates, while rough colonies were formed on modified Drigalski agar plates. The isolate was positive for catalase and oxidase tests, and other biochemical characteristics were determined using the commercially available bacterial identification kit, ID test NF-18 (Nissui Pharmaceutical, Tokyo, Japan). However, this kit failed to identify the isolate, resulting in profile number 000200. In detail, acid was not produced from D-glucose, fructose, maltose, D-galactose, D-xylose, D-mannitol, sucrose, and lactose. Hydrolyses of esculin, urea, and gelatin were negative. Citrate utilization test was positive. β-galactosidase, lysine decarboxylase, arginine dihydrolase, and ornithine decarboxylase tests were negative. Indole test and nitrate reduction test were negative. However, the isolate was identified to be *B. hinzii* by a MALDI Biotyper (Bruker Daltonics, Kanagawa, Japan) with an index of 2.250, which is considered optimal for species identification. For confirmation, the 16S rRNA and *gyrB* gene sequences were analyzed, as described by Neilan et al. (Neilan et al., 1997) and Yamamoto and Harayama (Yamamoto & Harayama, 1995), and similar sequences were searched using the Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The 16S rRNA showed the highest similarity to *B. hinzii* NCTC13200^T (100%; 1455/1455 bp); however, other *Bordetella* species such as *B. pseudohinzii*, *B. bronchiseptica*, *B. parapertussis*, *B. pertussis*, *B. trematum*, and *B. holmesii* showed high homology of more than 98.7% and could not be evidently distinguished. In particular, the *B. pseudohinzii* strain HI4681 showed similarity of 99.9% (1453/1455 bp); only 2 bases were different. However, *gyrB* gene sequence revealed the highest similarity to *B. hinzii* NCTC13200^T (100.0%; 1177/1177 bp), and secondly to the *B. pseudohinzii* strain HI4681 (95.7%; 1126/1177 bp). Accordingly, the isolate was identified as *B. hinzii*. An antimicrobial susceptibility test was performed with MicroScan WalkAway 96 plus and the NM1J panel (Beckman Coulter, Tokyo, Japan), which can be used for the MIC testing of gram-negative non-fermenting bacilli. Table 1 shows the susceptibility results of the isolate in the first sampling. The MICs of the isolates from the first, second, and third sampling were within two 2-fold dilutions, except for piperacillin, in which case the first isolate was susceptible to 8 μg/mL piperacillin, while the second and third isolates were resistant to >64 μg/mL of the antimicrobial. The antimicrobial susceptibility testing was interpreted using the MIC breakpoints for other non-Enterobacteriaceae in the Clinical and Laboratory Standards Institute guideline (Clinical and Laboratory Standards Institute (CLSI), 2019). The nitrocefin test was negative.

Table 1
Antimicrobial susceptibility testing.

Antibiotics	MIC (μg/mL)	Interpretation[a]
Ampicillin	>16	N/A
Piperacillin	≤8	S
Cefaclor	>16	N/A
Cefazolin	>16	N/A
Cefmetazole	>32	N/A
Cefotiam	>16	N/A
Flomoxef	<4	N/A
Ceftazidime	8	S
Cefdinir	>2	N/A
Cefcapene	>2	N/A
Cefpodoxime	>4	N/A
Cefotaxime	>2	N/A
Ceftriaxone	>2	N/A
Cefepime	16	I
Cefozopran	8	N/A
Ampicillin-sulbactam	>16/8	N/A
Amoxicillin-clavulanate	≤8/4	N/A
Piperacillin-tazobactam	≤8	S
Cefoperazone-sulbactam	≤16/8	N/A
Aztreonam	>16	R
Imipenem	≤1	S
Meropenem	≤1	S
Fosfomycin	>16	N/A
Ciprofloxacin	2	I
Levofloxacin	1	S
Sitafloxacin	≤1	N/A
Trimethoprim-sulfamethoxazole	≤2/38	S
Amikacin	16	S
Gentamicin	8	I
Tobramycin	>8	R
Minocycline	≤2	S

S = susceptible; I = intermediate; R = resistant; N/A = categorical interpretation not available.

[a] Antibiotic susceptibility data were interpreted using the Clinical and Laboratories Standards Institute M100-S29 (CLSI, 2019).

2. Discussion

This case of *B. hinzii* infection was novel because the infection site was a subcutaneous tissue. Moreover, *gyrB* gene sequence analysis proved useful to identify *B. hinzii* with satisfactory accuracy.

This case was an infection of subcutaneous tissue by *B. hinzii*, unlike previous cases, which were of pulmonary infection, cholangitis, endocarditis, or bacteremia. The patient neither suffered from trauma nor had travel history, as well as any underlying diseases such as diabetes. Regarding animal exposure, in addition to owning a dog, the patient revealed that he had seen and captured mice in his house often. Therefore, *B. hinzii* might have colonized the mice; however, the exact route of infection remained unclear in this case. According to other reports on *B. hinzii* infection, most of the cases had no avian exposure and transmission via birds was not proved (Arvand et al., 2004; Cookson et al., 1994; Fry et al., 2007; Funke et al., 1996; González et al., 2019; Hristov et al., 2008; Palacián Ruiz et al., 2013). Thus, poultry might not be the main infection source. Although *B. hinzii* is regarded as an opportunistic pathogen, it should be noted that a few cases were reported in immunocompetent individuals such as in our case (Kattar et al., 2000; Palacián Ruiz et al., 2013). More cases should be accumulated because the pathogenicity of *B. hinzii* is unclear.

The isolate had high MICs against many antimicrobials, including most cephalosporins, and had low MICs against carbapenems, trimethoprim-sulfamethoxazole, and fluoroquinolones, but not ciprofloxacin. Although the susceptibility pattern of *B. hinzii* against various antimicrobials differs depending on the strain, the isolates from this study and those reported previously in literature were all susceptible to piperacillin-tazobactam (Clinical and Laboratory Standards Institute (CLSI), 2019; Fabre et al., 2015; Palacián Ruiz et al., 2013). In addition, the isolates showed higher MICs for ciprofloxacin than for levofloxacin, as reported by previous studies (Fabre et al., 2015; Palacián Ruiz et al., 2013). There is a possibility that fluoroquinolones affect *B. hinzii* differently. In the present case, sitafloxacin seemed to be effective because the patient showed clinical improvement after initiating sitafloxacin administration. Before the treatment with sitafloxacin, the patient was administered cefditoren, amoxicillin-clavulanate, garenoxacin, and trimethoprim-sulfamethoxazole; however, the patient did not recover despite the fact that the isolate was susceptible to trimethoprim-sulfamethoxazole and showed low MIC against amoxicillin-clavulanate in vitro. The reasons for the ineffectiveness of these antimicrobials could be the dosing period and the penetration of the antimicrobial agents. Size reduction of the abscess was observed more than 3 weeks after administration of sitafloxacin was initiated; however, the dosing periods of amoxicillin-clavulanate and

trimethoprim-sulfamethoxazole were less than 2 weeks. In particular, considering that the target in the present case was an abscess, the penetration of antimicrobials might have been poor; it is possible that these antimicrobials might have been effective with longer administration. A previous report also mentions a case where *B. hinzii* could not be treated with trimethoprim-sulfamethoxazole or piperacillin-tazobactam, to which the isolate was susceptible, possibly because the treatment duration and curative doses of antimicrobial agents for *B. hinzii* infection are not standardized, leading to variations in effectiveness (Fabre et al., 2015). Therefore, an appropriate dosage of antimicrobials for *B. hinzii* infection needs to be established.

The biochemical identification kit, similar to other biochemical identification kits such as API20NE, failed to identify the isolate because *B. hinzii* is not included in the identification database of ID test NF-18 (Arvand et al., 2004; Cookson et al., 1994; Fabre et al., 2015; Fry et al., 2007; Funke et al., 1996; Kattar et al., 2000). Biochemical characterization of the isolate observed with the kit was similar to that reported for *B. hinzii* isolates in existing literature (Ko et al., 2005; Vandamme et al., 1996). 16S rRNA analysis is one of the most frequently used methods for bacterial identification; however, the 16S rRNA sequencing results of *B. hinzii* were similar to those of other *Bordetella* species, especially to *B. pseudohinzii*, which was recently identified and is the nearest phylogenetic neighbor of *B. hinzii* (Ivanov et al., 2016). It has been reported that MALDI-TOF-MS was effective in the identification of *B. hinzii* (Degand et al., 2008; Fabre et al., 2015; Palacián Ruiz et al., 2013); however, the reference spectrum of *B. pseudohinzii* is not registered in the MALDI Biotyper Reference library (version 8.0.0.0) for now, and it is unclear whether *B. hinzii* and *B. pseudohinzii* can be distinguished. However, identification with *gyrB* gene sequencing was obvious, suggesting that *gyrB* sequencing is a powerful tool for identification of *B. hinzii*. *B. hinzii* might have been overlooked in daily examination due to the difficulty in identification with phenotypic methods. Further investigation of *B. hinzii* is required to understand its virulence; hence, a reliable identification method including *gyrB* gene sequence analysis is necessary.

In conclusion, we present a case of subcutaneous abscess due to *B. hinzii* in an immunocompetent patient. Although it is rare, *B. hinzii* should be considered as a potential pathogen in immunocompetent individuals. It is important to correctly identify the species using *gyrB* gene sequencing to gather information about *B. hinzii* infection.

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Conflict of interest

The authors declare that there is no competing interest.

Compliance with ethical standards

All procedures performed in this study were in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

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