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Case report

Pasteurella bettyae infection requiring finger amputation due to rapid deterioration and tissue damage

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ABSTRACT

We report a case of infection of the middle finger of a 69-year-old man who visited our hospital. Pus was collected from the erythematous and swollen area of the nail cage of the left-hand middle finger and evaluated in our microbiology laboratory. Gram staining of the specimen revealed multinucleated leukocytes and abundant gram-negative bacilli. Isolated colonies were identified as *Pasteurella bettyae* using VITEK MS and 16 S ribosomal RNA (rRNA) gene sequencing. The patient's blood test results improved after treatment with penicillin, but the local factors affecting the finger did not improve, and amputation of the middle finger had to be performed. This case represents a report of a very rare hand infection caused by *P. bettyae*. Polymorphic identification methods, such as MALDI-TOF MS and 16 S rRNA gene sequencing, are needed for members of the genus *Pasteurella* isolated from severe infections and abnormal sites, and further studies are warranted.

Introduction

Pasteurella spp. are small Gram-negative bacilli found in the mucous membranes of the nasopharynx and other parts of wild and domestic animals, including pets such as dogs and cats, and are important opportunistic pathogens in humans [1,2]. Most human infections with *Pasteurella* spp. are through direct contact, for example being bitten or having a wound licked by an animal [3]. Among these species, *Pasteurella multocida* is most commonly isolated from human infections, and it is associated with serious conditions, such as bacteremia, meningitis, and endocarditis [1,2,4].

In contrast, *Pasteurella bettyae* is not often detected in human infections. Only five cases of *P. bettyae* infecting the pleura, blood, reproductive organ and urinary tract have been reported in PubMed [5–9]. Of those, only two reports have been published since 2000. This case represents a report of a very rare hand infection caused by *P. bettyae*.

Case report

Clinical findings

A 68-year-old man with no pre-existing medical conditions and no history of animal pet ownership or contact visited his physician, complaining of swelling of the tip of the left-hand middle finger since early June 2022. He was prescribed cefcapene pivoxil for 3 days (100 mg three times/day), but he visited our clinic in mid-June due to worsening of local findings (Figs. 1–1). After collecting pus from the swollen area of the nail cage and submitting it for microbiological examination, the patient was started on oral minomycin for 3 days (100 g twice/day) as a predictive dose, assuming it was a case of methicillin-resistant *Staphylococcus aureus* infection. The patient complained of further swelling and worsening of local initial findings and returned to the clinic 3 days later (Figs. 1–2). Blood test values at that time showed elevated white blood cell counts and C-reactive protein (CRP) inflammatory markers levels, as

Abbreviations: rRNA, ribosomal RNA; CRP, C-reactive protein; HbA1c, hemoglobin A1c; MIC, minimum inhibitory concentration; MALDI-TOF MS, matrix-assisted laser desorption-ionization time-of-flight mass spectrometry.

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Fig. 1. Patient's affected area findings. (1-A) At the time of the first visit to our clinic. Swelling around the nail cage was observed and skin discoloration was observed. (1-B) Three days after the initial visit. The swelling and discoloration area extended to the center of the finger and the local findings worsened.

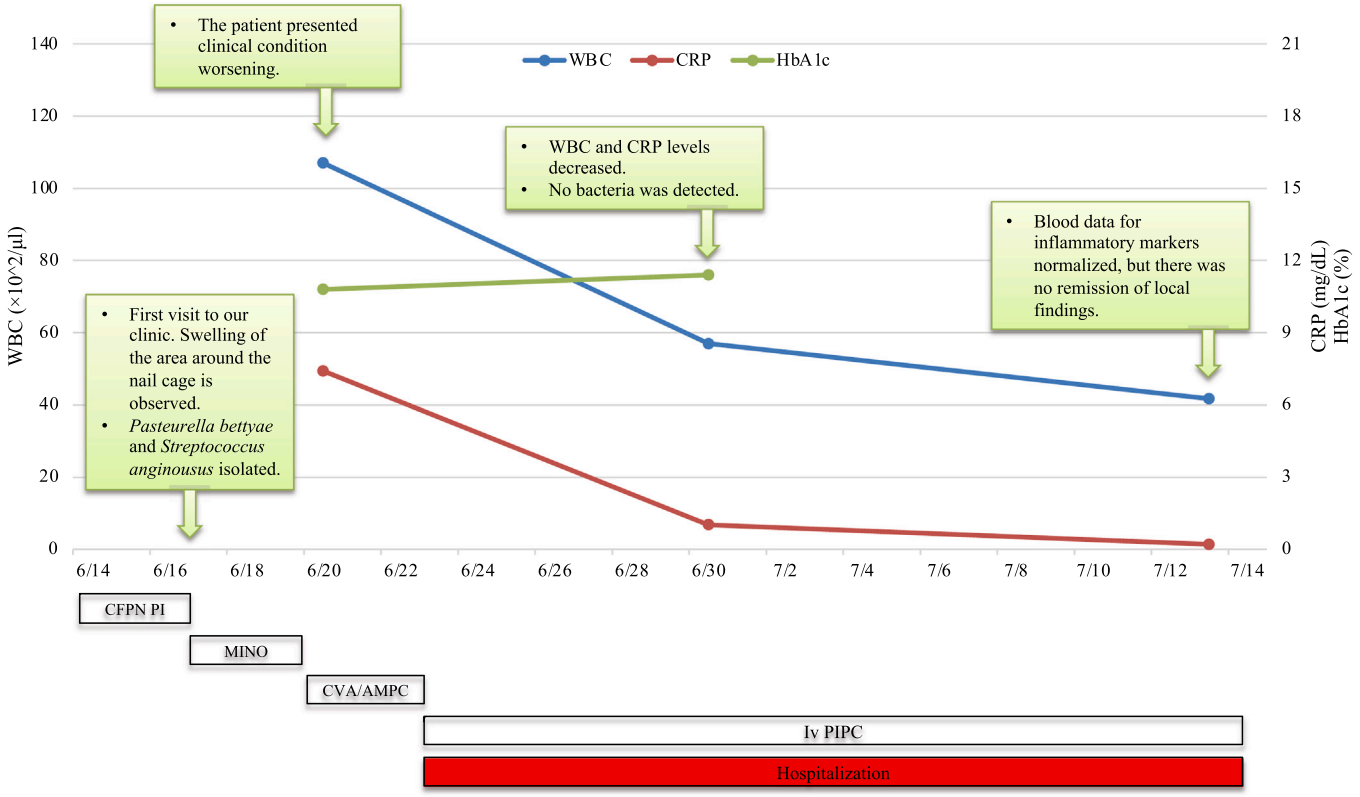

Fig. 2. White blood cell counts, C-reactive protein and hemoglobin A1c values based on the clinical course of the patient from June 14 to July 14, 2022.

Table 1
Main laboratory results at outpatient clinic.

WBC	107	$\times 10^2/\mu L$	AST	16	IU/L
Neu	80.3	%	ALT	21	IU/L
Lym	11.5	%	CRP	7.41	mg/dL
Mon	5.2	%	BUN	14.9	mg/dL
Eosino	1.3	%	CRE	0.66	mg/dL
Baso	0.3	%	HbA1c(N)	10.8	%
RBC	497	$\times 10^4/\mu L$			
Hb	14.9	g/dL			
Hct	42.7	%			
MCV	85.8	fL			
MCH	29.9	pg			
MCHC	34.9	g/dL			
Plt	23.9	$\times 10^4/\mu L$			

well as elevated hemoglobin A1c (HbA1c) (Table 1). Diabetes mellitus was also suspected. A large amount of *P. bettyae* and a small amount of *Streptococcus anginosus* were detected in the pus specimen from the initial visit, and bacterial infection was observed, so the patient was switched from minomycin to oral clavulanate/amoxicillin for 3 days (125 g/500 g three times/day) before the drug sensitivity results were available (Fig. 2). Thereafter, there was no improvement in the local findings, and the patient was admitted to the hospital for a close examination in late June.

Progress after hospitalization

On the day of admission, the patient's medication was switched to intravenous piperacillin (1 g twice/day). Microbiological tests performed during hospitalization returned negative, and blood test results showed improvements, including normalized white blood cell counts and decreased CRP; however, no improvement was detected in the necrotic-like findings of the left middle finger. The patient was transferred to a hospital in mid-July, where surgery was performed to amputate the middle finger from the proximal phalanx. The patient was discharged from the hospital in late July after the postoperative course was confirmed.

Bacteriological examination

Gram staining of the pus at the initial visit revealed large amounts of gram-negative bacilli and a few gram-positive cocci against a background of numerous polymorphonuclear leukocytes. The culture showed an abundance of *P. bettyae* (Figs. 3–1), as well as a small number of *S. anginosus*.

P. bettyae formed small gray colonies without hemolysis on blood agar (Becton Dickinson, Japan), larger gray colonies on chocolate agar medium than on blood agar (Becton Dickinson, Japan) (Figure 3–2), and smaller gray colonies on modified drigalski agar medium than on blood agar (Becton Dickinson, Japan) (Figs. 3–3). The isolate was subsequently identified as *P. bettyae* using the VITEK MS (bioMérieux, France) system and confirmed via 16 S ribosomal RNA (rRNA) gene sequencing using universal primers 27 F (5′-AGTTTGATCMTGGCTCAG-3′) and 1492 R (5′-TACGGGYTACCTTGTTACGACTT-3′) [10]. The sequence was found to be 99.85% (1332/1334 bp) identical to *P. bettyae* type strain CCUG 2042 (GenBank accession number NR_042880) using the NCBI 16 S rRNA gene database. A biochemical identification test using the GN panel of VITEK2 (bioMérieux, France), a conventional method, was performed, and the strain was determined to be split into two species, *Pasteurella canis* and *Pasteurella multocida,* with equal probabilities (BioNo: 0001010000040000).

Susceptibility to 13 antimicrobial agents was determined using dry plates (Eiken Chemical, Japan). The minimum inhibitory concentration (MIC) breakpoints used in this study were those established by the Clinical and Laboratory Standards Institute [11] for the genus *Pasteurella*. The MICs for *P. bettyae* isolates are shown below. The MICs for

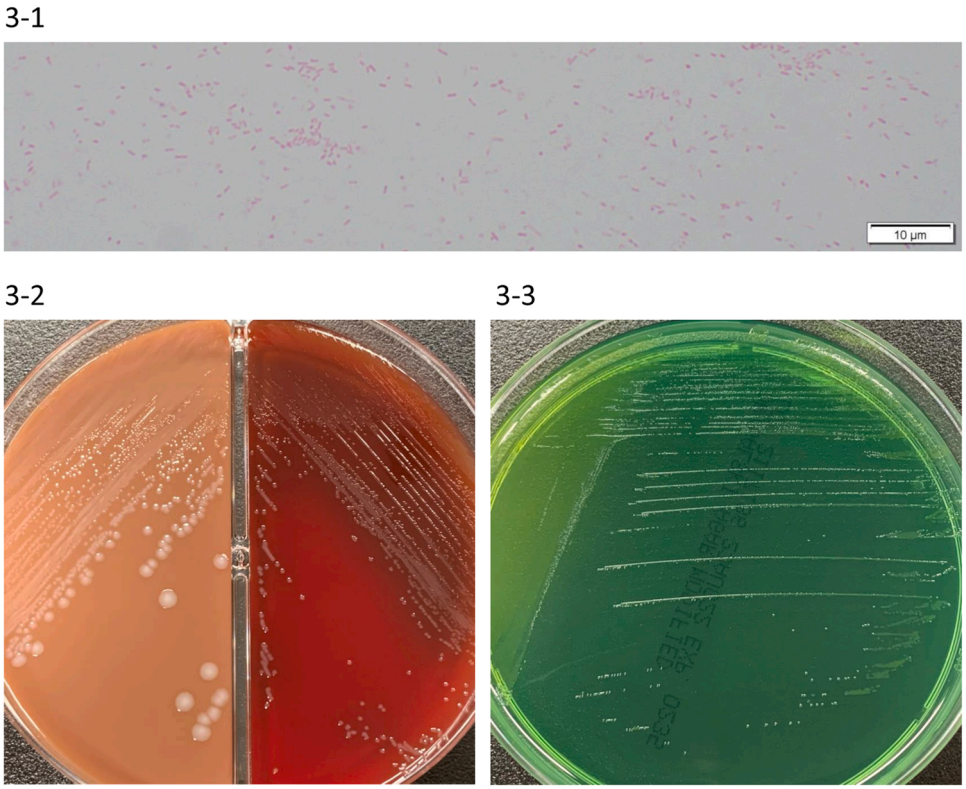

Fig. 3. Phenotypic characteristics of *Pasteurella bettyae*. (3-A) *P. bettyae* appeared as gram-negative bacilli in short chains upon Gram staining. (3-B, C) On blood agar and modified Drigalski agar, the *P. bettyae* isolates appeared as gray, small colonies. larger gray colonies on chocolate agar medium than on blood agar and modified Drigalski agar.

Table 2
Clinical features of reported cases of *Pasteurella bettyae* (Source: PubMed, Medline).

Year of publication	Reference number	Authors	Patient age (years)	Gender	Diabetes mellitus	Clinical condition	Source	Polymicrobial/ monomicrobial	Isolated species	Identification method	Antibiotic treatment	Outcome
2021	9	Rosales-Castilloa A et al.	53	Male	-	HIV-positive	Urethral exudate	Monomicrobial	*Pasteurella bettyae*	MALDI-TOF MS (Bruker MALDI BioTyper system)	Oral doxycycline (21 days)	Unknown
			47	Male	-	HIV-positive	Urethral exudate	Monomicrobial	*Pasteurella bettyae*		Oral ceftriaxone (1 day) and doxycycline (28 days)	Unknown
2015	8	Gómez-Camarasa C et al.	69	Male	-	Prostate cancer	Urine	Monomicrobial	*Pasteurella bettyae*	API NF system, MALDI-TOF MS (Bruker MALDI BioTyper system)	Unknown	Unknown
1996	7	Shapiro DS et al.	25	Female	-	Pregnancy	Blood	Polymicrobial	*Pasteurella bettyae*, *Veillonella parvula*	Unknown	Iv ampicillin and gentamycin	Unknown
1996	6	Moritz F et al.	40	Male	-	HIV-positive	Pleural fluid	Monomicrobial	*Pasteurella bettyae*	Unknown	Iv piperacillin-tazobactam, ciprofloxacin and amikacin	Died
1989	5	Baddour LM et al.	23	Female	-	Vaginal bleeding	Cervical swab	Unknown	CDC group HB-5 (*Pasteurella bettyae*)	Unknown	Iv ceftriaxone, Oral metronidazole (1 day) and doxycycline (10 days)	Recovered
			20	Male	-	Yellow penile discharge	Yellow penile discharge	Unknown	CDC group HB-5 (*Pasteurella bettyae*)	Unknown	Iv ceftriaxone, and oral tetracycline (5 days)	Recovered
			30	Female	-	Lower abdominal pain	Vaginal swab	Unknown	CDC group HB-5 (*Pasteurella bettyae*)	Unknown	Iv ceftriaxone, and oral tetracycline	Recovered
			25	Female		Abdominal pain	Cervical swab	Polymicrobial	CDC group HB-5 (*Pasteurella bettyae*), Normal flora	Unknown	Iv penicillin	Recovered
			38	Female		Boil of the external genitalia	Bartholin gland	Unknown	CDC group HB-5 (*Pasteurella bettyae*)	Unknown	-	Recovered

amoxicillin 0.25, amoxicillin/clavulanate ≤ 0.25, ampicillin ≤ 0.12, penicillin 0.25, ceftriaxone ≤ 0.06, doxycycline ≤ 0.5, tetracycline ≤ 1, azithromycin 0.25, chloramphenicol ≤ 2, and sulfamethoxazole/-trimethoprim ≤ 10 were all sensitive, while erythromycin > 2 was resistant. Levofloxacin ≤ 0.5 and moxifloxacin ≤ 0.5 could not be interpreted because they were outside the range.

Discussion

P. bettyae is a Gram-negative, non-motile bacillus belonging to the Pasteurellaceae family. Unlike other *Pasteurella* spp, *P. bettyae* is catalase-test negative and oxidase-test variable [12]. In addition, the natural habitat of *P. bettyae* is unknown, although many *Pasteurella* spp. have been isolated from the oropharyngeal bacteria of dogs and cats [13]. In humans, pasteurellosis is most commonly caused by *P. multocida*, which is typically transmitted to the skin and soft tissues after animal bites. *P. bettyae* has not been reported as an infectious disease of zoonotic origin, but various infections of pleurisy, bacteremia in pregnant women, reproductive organ origin, and those of urinary origin have been reported in the old days (Table 2). Although not listed in PubMed, the same finger infection as this case was reported by De Leon JP et al. [14]. Since 2000, urinary and genitourinary infections with *P. bettyae* have been reported, suggesting a link [8,9]. Previous publications on cases with *P. bettyae* also indicated an association of the bacteria with immunocompromised indiviuals, including HIV-positive patients [6,9]. Our patient had no history of pet ownership, animal contact, or sexual activity within 10 years, and the cause of infection was unknown. This patient did not have HIV, but was suspected to have diabetes, making him susceptible to infection. Although past reports indicate that *P. bettyae* does not cause serious infections [6], this presumptive diabetic condition may have contributed to the lack of improvement in the local findings in this case, despite improved blood results after treatment. In addition, a small number of *S. anginousus* was identified in this case, along with *P. bettyae*. Diabetes mellitus has been reported to be involved in 25% of *S. anginousus* infections [15]. It is possible that *S. anginousus*, together with *P. bettyae*, may have contributed to the severity of the disease in this case, in which diabetes mellitus is presumed.

P. bettya is microbiologically similar to other *Pasteurella* species, although most *Pasteurella* species are catalase positive, while *P. bettyae* is negative. In the present case, the organisms were found to form gray colonies on blood agar medium, and the results were consistent even with catalase negativity [12]. Another differentiating feature of *P. bettyae* is that all *Pasteurella* spp. are saccharose positive other than *P. bettyae,* which is saccharose negative [12]. The introduction of molecular and genetic identification methods for microorganisms has enabled the detection of new and rare bacteria in clinical specimens. Methodologies, such as matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) and 16 S rRNA gene sequencing, are effective for identifying *P. bettyae* [8,9]. Use of biochemical identification, such as the GN panel of VITEK2, is not very useful for *P. bettyae* identification because its information is not included in the database. We emphasize the usefulness of MALDI-TOF and gene sequencing for this organism, and if these identification methods are adopted more frequently in clinical laboratories, more frequent identification of rare bacteria may clarify the epidemiological background of the bacteria.

Pasteurella species have been reported to be susceptible to penicillins (penicillin G, amoxicillin-clavulanate, piperacillin), fluoroquinolones (levofloxacin, moxifloxacin), cephalosporins (ceftriaxone, cefixime, cefpoxime), doxycycline, and carbapenems [13]. Treatment failures have also been reported, and the use of oral macrolides (erythromycin), oxacillin, dicloxacillin, first-generation cephalosporins, and clindamycin should be avoided [16]. This is consistent with the susceptibility profile of *P. bettyae* in this case. *P. bettyae* infections are reported to have been treated with penicillins, cephalosporins, and doxycycline [5–9]. Although erythromycin was used in the treatment of a previously reported finger infection, penicillin drugs improved the blood data in this case [14]. This provides evidence for the contribution of this organism to this patient's condition.

In summary, we report the detection of *P. bettyae* in a human clinical specimen. Such rare microorganisms are difficult to identify using conventional methods. Therefore, polymorphic identification methods such as MALDI-TOF MS and 16 S rRNA gene sequencing are needed for members of the genus *Pasteurella* isolated from severe infections and abnormal sites, and further studies are warranted. We believe that early bacterial nomenclature reporting will provide a means of clinical management based on previous case reports.

Ethics approval to participate

Ethics approval for this case report were waived.

Authors' contributions

Daisuke Kitagawa wrote the first draft of the manuscript. Anna Ochi and Toru Kurimoto were the attending physicians. Takehito Kasamatsu and Naoyuki Shiraishi assisted with patient care. Soma Suzuki, Yui Shintani and Madoka Furumori offered the measuring microbiological data. Yuki Suzuki, Akiyo Nakano and Ryuichi Nakano performed the genetic analysis of the bacteria. Hisakazu Yano and Koichi Maeda revised all versions. Kyoko Nomi and Fumihiko Nakamura supervised the process of drafting the manuscript. All authors have read and approved the final manuscript.

Consent for publication

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

Authorship statement

All authors meet the ICMJE authorship criteria. Daisuke Kitagawa drafted the initial manuscript. All authors critically reviewed the manuscript and approved submission of the final version.

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Declaration of Competeing Interest

None.

Data Availability

All data generated or analyzed during this study are included in this published article.

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