Bacteremia due to *Pasteurella dagmatis* acquired from a dog bite, with a review of systemic infections and challenges in laboratory identification

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A case of bacteremia in a 74-year-old man, which was caused by *Pasteurella dagmatis* and complicated by thrombocytopenia, is presented. Microorganism identification was performed by the provincial reference laboratory using traditional biochemical profiling, completmented with both the sequencing of the 16S ribosomal RNA gene and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; antibiotic-susceptibility testing was also performed. After treatment with the appropriate antibiotics, the patient fully recovered. Systemic infections attributed to this organism are rarely reported in the literature. Other reported cases of bacteremia due to *P dagmatis* are reviewed and compared with the present case. The challenges of relying on standard automatic identification are discussed, with alternative methodologies provided.

Key Words: *16S rRNA gene sequencing; Bacteremia; Dog bite; Identification; MALDI-ToF MS;* Pasteurella dagmatis; *VITEK 2*

Une bactériémie à *Pasteurella dagmatis* contractée par une morsure de chien, une analyse des infections systémiques et les difficultés pour le dépistage en laboratoire

Les auteurs présentent un cas de bactériémie chez un homme de 74 ans, causé par un *Pasteurella dagmatis* et compliqué par une thrombocytopénie. Le laboratoire de référence provincial a identifié le microorganisme au moyen du profilage biochimique classique et l'a complété par le séquençage du gène de l'ARN ribosomique 16S et par la spectrométrie de masse à temps de vol par désorption-ionisation laser assistée par matrice. Le laboratoire a également effectué un test de susceptibilité aux antibiotiques. Après un traitement antibiotique pertinent, le patient s'est complètement rétabli. Les publications scientifiques contiennent peu de déclarations d'infections systémiques attribuées à cet organisme. D'autres cas de bactériémie à *P dagmatis* sont analysés et comparés à la présente situation. Les problèmes liés à l'identification automatique standard sont exposés et d'autres méthodologies sont proposées.

CASE PRESENTATION

A 74-year-old man startled his dog and sustained a penetrating bite to his hand. The dog then licked the blood off the injured extremity. A few days later, the patient developed fever, chills and weakness, resulting in a fall. He presented to the emergency room for assessment. Medical history and significant comorbidities included chronic obstructive pulmonary disease, hypertension, dyslipidemia and idiopathic dilated cardiomyopathy, Grade 3 left ventricle. No immunocompromising illnesses were present. Physical examination revealed a temperature of 40°C, with bronchial breath sounds and crackles heard in the left chest; the bite wound appeared improved. A chest x-ray revealed a left perihilar infiltrate. Peripheral white blood cell count was 15.5×10^9/L, neutrophils 13.4×10^9/L, hemoglobin 126 g/L and platelet nadir $14{,}000\times10^9$/L. The patient was admitted for further investigation and treatment. Blood cultures grew coagulase-negative *Staphylococcus*, which was considered to be a contaminant, and Gram-negative coccobacilli were later determined to be *Pasteurella dagmatis*. Treatment was initiated with oral azithromycin 500 mg per day and intravenous ceftriaxone 1 g every 24 h for five days. The patient defervesced within four days and his condition improved dramatically. Oral levofloxacin 500 mg was administered as step-down therapy for 10 days. The hematological abnormalities resolved. On follow-up three months later, he remained well.

Laboratory findings

Two sets of blood cultures were drawn 5 h arpart from the patient on the day of admission using BD BACTEC PLUS aerobic/F and anaerobic/F bottles (Becton, Dickinson and Company, Canada). Three bottles produced Gram-negative coccobacilli with beaded ends in 14 h to 34 h, and one anaerobic/F bottle also produced Gram-positive cocci in clusters, which were subsequently identified as coagulase-negative staphylococci; this organism was considered to be a contaminant. The subcultures on blood agar plates produced tiny grey-brown creamy colonies, which did not grow on MacConkey agar. The Gram-negative coccobacilli were initially identified as *Pasturella pneumotropica* by the VITEK 2 system, software version 06.01 (BioMerieux, France) using the GN card, with bionumber 0001010210040001 and an excellent identification (probability 99%). Unusual bacteria such as this are routinely sent to the local reference laboratory (Public Health Ontario, Toronto, Ontario) for confirmation of identification and susceptibility testing. The susceptibility profile of the bacterium was interpreted by CLSI M45-A2 (1) (Table 1).The biochemical characteristics (Table 2), 16S ribosomal RNA (rRNA) gene polymerase chain reaction (PCR) and sequencing (below), as well as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS) were used for identification of the bacterium.

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TABLE 1
Antibiotic susceptibility of *Pasteurella dagmatis*

Antibiotic	Mininmum inhibitory concentration, mg/L	Interpretation
Penicillin	0.12	Sensitive
Ampicillin	≤2	Sensitive
Pipercillin/tazobactum	≤4	Sensitive
Cefazolin	≤4	Sensitive
Ceftazidime/ceftriaxone	≤1	Sensitive
Meropenem	≤0.25	Sensitive
Gentamicin/tobramycin	≤1	Sensitive
Ciprofloxacin	≤0.25	Sensitive
Levofloxacin	≤0.03	Sensitive
Trimetoprim/sulfamethoxazole	≤20	Sensitive

Analysis performed using VITEK 2 (BioMerieux, France) and agar dilution as per the Clinical and Laboratory Standards Institute (1)

TABLE 2
Key biochemical characteristics of *Pasteurella dagmatis*, *P stomatis* and *P pneumotropica*

	P dagmatis	*P stomatis*	*P pneumotropica*
Dextrose	+	+	n/a
Lactose	–	–	–
Sucrose	+	+	+
Xylose	–	–	+
Mannitol	–	–	–
Maltose	+	–	+
Arabinose	–	–	V
Sorbitol	–	–	–
Trelose	+	+	+
Dulcitol	–	–	–
Catalase	+	+	+
Oxidase	+	+	+
TSI slant/butt	+/+	+/+	+/+
Indole	+	+	+
Urea activity	+	–	+
Nitrate to nitrite	+	+	+
Motility	–	–	–
Ornithine	–	–	+
Arginine	–	–	n/a
Lysine	–	–	V
Ortho-nitrophenyl-β-galactoside	–	–	+

Data presented as positive (+) or negative (–). Reactions refer to reference 2. n/a Not available; V Variable;

Biochemical profile
Traditional biochemical testing was performed on the isolate and based on its profile was determined to be *P dagmatis* (2) (Table 2). Because this organism is not often encountered, alternate identification methods were also used to ensure a correct identification.

16S rRNA gene PCR/sequencing
16S rRNA gene PCR was performed at Public Health Ontario. A 736-base pair amplicon was generated (primers, forward: 5'–AGTTTGATCCTGGCTCAG–3'; Reverse: 5'–GGACTACCAGGGTATCTAAT–3') and sequenced using routine methods (3). The sequence was analyzed using National Center for Biotechnology Information basic local alignment search tool (4) and results were interpreted using CLSI MM18-A guidelines (5). The PCR product was 99% similar to six deposits within the nr/nt database with 99% to 100% coverage. Sequences with high levels of homology to the query sequence included the type strain of *P dagmatis*, ATCC 43325/CCUG 12397 (99%; NR_042883.1 and M75051.1) and the type strain of *Pasteurella stomatis*, CCUG 17979 (99%; NR_042888.1). Based on CLSI MM18-A interpretation guidelines (5), due to the low level of demarcation of the sequence of the 16S rRNA gene between these species, the unknown bacteria may only be identified as *P dagmatis* or *P stomatis*. However, based on the biochemical profile (Table 2), this organism could not be *P stomatis* (which is urease and maltose negative, because the organism in question is urease and maltose positive); therefore, in the present case, the identification of the organism was *P dagmatis*.

TABLE 3
Summary of reports involving bacteremia in humans with *Pasteuralla dagmatis* in the English literature

Patient age, years/sex	Animal bites	Diagnosis	Antibiotic Treatment	Outcome	Reference
78/male	None	Prosthetic valve endocarditis	Ceftriaxone	Survived	10
66/male	None	Septicemia	Penicillin	Recovered	11
51/male	Dog	Septicemia, diabetic foot	Penicillin	Resolved	12
55/female	Cat	Infectious endocarditis thrombocytopenia	Ceftriaxone	Survived	14
77/male	Cat	Prosthetic valve endocarditis	Ceftriaxone, penicillin	Recovered	15
56/female	Dog	Peritonitis, septicemia, cirrhosis	Benzypenicillin, ciprofloxacin, metronidazole	Passed away within 24 h	16

MALDI-ToF MS
Single colonies of fresh organisms grown overnight were prepared using a modified formic acid extraction procedure and analyzed using the Bruker MALDI BioTyper (Bruker Daltonics, Germany) in duplicate using standard settings. The query spectra had a high level of similarity >2.0 (≥2.0 is an acceptable score for species-level identification) to *P dagmatis* spectra within the routine commercial database. The top five matches were to spectra from different strains of *P dagmatis* within the commercial database.

DISCUSSION AND LITERATURE REVIEW

P dagmatis is a relatively new species for many clinicians. It is a Gram-negative coccobacillus belonging to the *Pasteurellaceae* family, which is fermentative, aerobic, nonmotile, oxidase positive and penicillin-sensitive. This organism has been isolated from both dogs and cats as normal flora, and also reported as a pathogen in human infections. It was previously labeled as *Pasteurella* "gas", *Pasteurella* new species 1 or *P pneumotropica* type Henriksen, and was eventually reclassified as *P dagmatis* (6).

Bacteria from the *Pasteurellaceae* family cause zoonotic infections in humans, with *P multocida* and *P canis* being the most common *Pasturella* species reported in human infections (7,8). Infections caused by *Pasteurella* species are typically introduced by animals, particularly cat or dog bites, but also occasionally by other animals, and often manifest as skin or soft tissue infections (7-9). Sometimes, animal contact is not prominent in the initial patient history (10,11) (Table 3). The most probable route of transmission of *P dagmatis* infection in the present case was most likely the bite and licking of the patient's traumatized skin by his dog, as has been previously described (11,12). Continuous shedding of *P dagmatis* from asymptomatic animals (eg, in dog urine [13]) and whether it can be an indirect route of infection to human remains to be investigated.

While *Pasteurella* species are well recognized for causing skin or soft tissue infections, *P dagmatis* can also cause more serious disease, including infective and prosthetic valve endocarditis (10,14,15), septicemia (11,12,16), peritonitis (17), vertebral osteomyelitis (18,19), chronic bronchiectasis (20) and pneumonia (21), mainly in immunocompromised patients. A small number of case reports describing systemic human *P dagmatis* infections are listed in Table 3. Interestingly, while *Pasturella* species infrequently cause systemic infectious disease, in our review of the literature, when *P dagmatis* infections are reported, they appear to be frequently associated with severe disseminated infection including bacteremia. Coinfections of *P dagmatis* with another *Pasturella* species have also been observed (9,12,22); therefore, it is important for the laboratory to test multiple morphotypes from the plate to ensure that >1 *Pasturella* species is not present.

Similar to other *Pasturella* species, *P dagmatis* is typically highly susceptible to many antibiotics, particularly, the beta-lactams (Table 1). Early suspicion and timely laboratory diagnosis of *Pasturella* infection are crucial for a favourable clinical outcome.

Several reports have demonstrated that the VITEK 2 GN card misidentifies *P dagmatis* as *P pneumotropica* or *P canis*, despite an excellent identification probability (9,15,23,24). This is most likely because *P dagmatis* has not been included in the system database; as well, there has been a nomenclature change because *P dagmatis* was formerly grouped with *P pneumotropica*, type Henriksen. In a study that included 66 clinical *Pasturella* isolates and used *sodA* gene sequencing as a reference method, Zangenah et al (24) revealed that VITEK 2 only identified approximately 50% of *Pasturella* isolates correctly, while conventional biochemical tests and MALDI-ToF MS were able to correctly identify 94% and 89%, respectively. Interestingly, in the Zangenah et al (24) study, two *P dagmatis* isolates were not identified by VITEK MS MALDI-ToF (BioMerieux, France) and this limitation was also observed in our study (data not shown). The biological and genetic profiles among *P dagmatis*, *P pneumotropica* and *P stomatis* are very similar (Table 2); both a commercial biochemical identification system and the sequence analysis of a portion of the 16S rRNA gene were unable to differentiate between these species. Correct identification was made using MALDI-ToF MS (MALDI Biotyper, Bruker, Germany) and was also supported by comparing the key biochemical characteristics among *P dagmatis*, *P pneumotropica* and *P stomatis* (Table 2). It is probable that many clinical isolates of *P dagmatis* have been misidentified due to the limitation of commercial biochemical identification systems, such as VITEK 2. Misidentification may have contributed to an underestimation of the frequency of this organism in clinical samples; however, the growing use of MALDI-ToF MS systems for microorganism identification in routine clinical microbiology laboratories may allow for a more accurate picture of how frequently *P dagmatis* causes infections. Correct identification is important for diagnosis and therapeutic management, and epidemiological monitoring of the transmission of *Pasturella* species, particularly for the systemic infections such as in the present case. Unfortunately, most routine methods available at hospital laboratories cannot identify the organism correctly.

CONCLUSION

P dagmatis can cause severe animal-associated infections in humans, mainly in immunocompromised individuals. To our knowledge, this is the first systemic *P dagmatis* infection reported in Canada. Clinical outcomes rely on early accurate laboratory confirmation and timely administration of effective antibiotic treatment. Conventional identification of *P dagmatis* using VITEK 2 can be misleading, probably due to the absence of this organism from the database; 16S rRNA gene sequence analysis and MALDI-ToF MS systems represent excellent options for identifying rarely encountered or difficult to identify organisms, such as members of the *Pasturellaceae* family. The present study re-emphasizes the need for continuously improving the database of automatic microbial identification systems.

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