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, complemented 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

Laboratory findings
Two sets of blood cultures were drawn 5 h apart 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 Pasteurella pneumotropica by the VITEK 2 system, software version 06.01 (BioMerieux, France) using the GN card, with biomonumber 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.
TABLE 1

Antibiotic susceptibility of *Pasteurella dagmatis*

| Antibiotic            | Minimum inhibitory concentration, mg/L | Interpretation |
|-----------------------|----------------------------------------|----------------|
| Penicillin            | ≤0.12                                  | Sensitive      |
| Ampicillin            | ≤2                                    | Sensitive      |
| Piperocillin/tazobactum | ≤4                                  | Sensitive      |
| Cefazolin             | ≤4                                    | Sensitive      |
| Ceftazidime/ceftiraxone | ≤4                                  | Sensitive      |
| Meropenem             | ≤0.25                                 | Sensitive      |
| Gentamicin/tobramycin | ≤4                                    | Sensitive      |
| Ciprofloxacin         | ≤0.03                                 | Sensitive      |
| Levofloxacin          | ≤0.03                                 | Sensitive      |
| Trimethoprim/sulfamethoxazole | ≤0.20                              | 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             | –             | –             | –                  |
| Treholose            | +             | +             | +                  |
| Dulcitol             | –             | –             | –                  |
| Catalase             | +             | +             | +                  |
| Oxidase              | +             | +             | +                  |
| TSI slant/butt       | +/+           | +/+           | +/+                |
| Indole               | +             | +             | +                  |
| Urea activity        | +             | +             | +                  |
| Nitrate to nitrite   | +             | +             | +                  |
| Motility             | –             | –             | –                  |
| Ornithine            | –             | –             | –                  |
| Arginine             | –             | –             | n/a                |
| Lysine               | –             | –             | V                  |
| Ortho-nitrophenyl-ß-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’–GGACTACCCAGGGTATCTAA–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/nr 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 a 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*.

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 (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 classified 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 *Pasteurella* 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 lancing 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 *Pasteurella* 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 *Pasteurella* 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 *Pasteurella* species is not present.

Similar to other *Pasteurella* species, *P. dagmatis* is typically highly susceptible to many antibiotics, particularly, the beta-lactams (Table 1). Early suspicion and timely laboratory diagnosis of *Pasteurella* 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 *Pasteurella* isolates and used sodA gene sequencing as a reference method, Zangenah et al (24) revealed that VITEK 2 only identified approximately 50% of *Pasteurella* 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.

**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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**REFERENCES**

1. Clinical and Laboratory Standards Institute. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; approved guideline – second edition. CLSI document M45-A2. Wayne: 19087-1898. Clinical and Laboratory Standards Institute; 2010.

2. Weyant R, Moss CW, Weaver RE, et al. Identification of unusual pathogenic Gram-negative aerobic and facultatively anaerobic bacteria. In: Hensley WR, 2nd edn. Philadelphia: Lippincott Williams and Wilkins, 1996:445-67.

3. Knox M, Cefallos V, and Dean D. 16S ribosomal DNA typing for identification of pathogens in patients with bacterial keratitis. J Clin Microbiol 1996;34:492-6.

4. Aitshul SF, Gish W, Miller W, Myers EW, Liptman DJ. Basic local alignment search tool. J Mol Biol 1990;215:403-10.

5. Clinical and Laboratory Standards Institute. Interpretive criteria for identification of bacteria and fungi by DNA target sequencing; approved guideline – first edition. CLSI document MM18-A. Wayne: 19087-1898. Clinical and Laboratory Standards Institute; 2008.

6. Mutters R, Ihm P, Pohl S, Frederiksen W, Mannheim W. Reclassification of the genus *Pasturella* trevisan 1887 on the basis of desoxyribonucleic acid homology, with proposals for the new species *Pasteurella dagmatis*, *Pasteurella canis*, *Pasteurella stomatis*, *Pasteurella anatis*, and *Pasteurella langii*. Int J Syst Bacteriol 1985; 35:309-22.

7. Abrahamian FM, Goldstein EJC. Microbiology of animal bite wound infections. Clin Microbiol Rev 2011;24:231-46.

8. Talan DA, Citron DM, Abrahamian FM, Moran GJ, Goldstein EJC. Bacteriological analysis of infected dog and cat bites. N Engl J Med 1999;340:85-92.

9. Akahane T, Nagata M, Matsumoto T, et al. A case of wound dual infection with *Pasteurella dagmatis* and *Pasterella canis* resulting from a dog bite – limitations of Vitek-2 system in exact identification of *Pasteurella* species. Eur J Med Res 2011; 16:531-6.

10. Rosenberg KA, Poblete J, Larkin I. Prosthetic valve endocarditis caused by *Pasteurella dagmatis*. South Med J 2001;94:1033-5.

11. Deschulder I, Gords B, Van Landuyt H, Renders F, Selleslag D. *Pasteurella dagmatis* septicaemia in an immunocompromised patient without a history of dog or cat bites. Acta Clin Belg 2000;55:225-6.

12. Fajfar-Whetstone CJ, Coleman L, Biggs DR, Fox BC. *Pasteurella multocida* septicaemia and subsequent *Pasteurella dagmatis* septicaemia in a diabetic patient. J Clin Microbiol 1995;33:202-4.

13. Mosallanejad B, Aveheh R, Ghadiri AR, Naddaf H, Jamshidian M. First report of *Pasteurella dagmatis* isolation from a bitch urine in Iran. Iran J Vet Res 2008;9:394-6.

14. Sobelbe AF, O’Donnell J, Kaiser-Smith J, Ficharris J, Shinkarow J, Doneson S. Infective endocarditis due to *Pasteurella dagmatis*: Case report and review. Clin Infect Dis 1994;18:336-8.

15. Strahm C, Goldenberger D, Gutmann M, Kuhnert P, Graber P. Prosthetic valve endocarditis caused by *Pasteurella dagmatis*-like isolate originating from a patient’s cat. J Clin Microbiol 2013;50:2818-9.

16. Ashley BD, Noone M, Dwarkanath AD, Malnick H. Fatal *Pasteurella dagmatis* peritonitis and septicaemia in a patient with cirrhosis: A case report and review of the literature. J Clin Pathol 2004;57:210-2.

17. Wallet F, Toure F, Devalkenaere A, Pagniez D, Courcol RJ. Molecular identification of *Pasteurella dagmatis* peritonitis in a patient undergoing peritoneal dialysis. J Clin Microbiol 2000;38:4681-2.

18. Garcia-Heij C, Biguillon C, Garcia C, et al. *Pasteurella dagmatis*: An unusual cause of vertebral osteomyelitis. J Pathobiol 2007;55:340-2.

**Pasturella dagmatis** bacteremia 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 *Pasteurella* 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.
19. Dupuy O, Garrabe E, Bordier L, et al. *Pasteurella dagmatis* spondylodiscitis in a diabetic patient. Revue de Medecine Interne 2006;27:803-4.

20. Allison K, Claridge JE. Long-term respiratory tract infection with canine-associated *Pasteurella dagmatis* and *Neisseria canis* in a patient with chronic bronchiectasis. J Clin Microbiol 2005;43:4272-4.

21. Laurens C, Marouze N, Jean-Pierre H. *Staphylococcus pseudintermedius* and *Pasteurella dagmatis* associated in a case of community-acquired pneumonia. Medicine et Maladies Infectieuses 2012;42:129-31.

22. Zbinden R, Sommerhalder P, von Wartburg U. Co-isolation of *Pasteurella dagmatis* and *Pasteurella multocida* from cat-bite wounds. Eur J Clin Microbiol & Infect Dis 1988;7:203-4.

23. Guillard T, Duval V, Jobart R, et al. Dog bite wound infection by *Pasteurella dagmatis* misidentified as *Pasteurella pneumotropica* by automated system Vitek 2. Diag Microbiol and Infect Dis 2009;65:347-8.

24. Zangenah S, Güleryüz G, Borång S, Ullberg M, Bergman P, Ozcenci V. Identification of clinical *Pasteurella* isolates by MALDI-TOF – a comparison with VITEK 2 and conventional microbiological methods. Diag Microbiol and Infect Dis 2013;77:96-8.