Case Report

Diabetic Foot Infection with *Bacteroides pyogenes*

Padmaja Ananth Shenoy, Shashidhar Vishwanath, Ravikumar Terikere Nagaraj, Barnini Banerjee, M. Sunil Krishna[1]

Departments of Microbiology and [1]Surgery, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, Karnataka, India

Abstract

Diabetic foot infections are the most common serious diabetes-related complication posing significant socioeconomic burden on the health-care system. Diabetic foot microbiota consists of polymicrobial flora with predominance of Gram-negative aerobes and anaerobes. Here, we report a rare case of diabetic foot infection by *Bacteroides pyogenes*, an obligate Gram-negative anaerobic bacillus which is commonly encountered in polymicrobial animal bite wound infections.

Keywords: Animal bite wound, *Bacteroides pyogenes,* diabetic foot infection, Gram-negative anaerobic bacilli

Introduction

Diabetes mellitus represents major health problem worldwide with diabetic foot infections being the most common complication necessitating hospital admissions.[1] The severe forms of diabetic foot infections are polymicrobial in nature with involvement of both aerobic (*Klebsiella* spp., *Escherichia coli*, and *Staphylococcus aureus*) and anaerobic (*Bacteroides* spp. and anaerobic streptococci) microflora.[1] The genus *Bacteroides* consist of Gram-negative, nonsporing anaerobic rods that form the integral part of the colonic microflora in humans. *Bacteroides* spp. are the frequently isolated anaerobes, accounting for more than one-third of the clinical specimens received in anaerobic bacteriology laboratory annually.[2] *Bacteroides pyogenes* is a normal inhabitant of oral flora of dogs and is gaining its clinical importance in recent years.[3] Among the *Bacteroides* group, *B. pyogenes* and *Bacteroides tectus* are closely related species encountered in dog and cat bite infections.[4-6] Here, we report a case of an infected diabetic ulcer without any prior history of animal bite.

Case Report

A 55-year-old daily wage worker presented to the department of surgery with complaints of fever and an ulcer over the left foot of 2 days' duration which was associated with foul smelling discharge. On local examination, the 2 cm × 2 cm ulcer was situated on plantar aspect of the left foot with the floor of the ulcer covered with slough. Bluish discoloration and wet gangrene of the fourth toe were also noted. There was local rise of temperature in the left foot and with no associated tenderness. All the peripheral pulses were felt. The patient was a known diabetic since 20 years and was on medication. Two years before this presentation, he was admitted for the complaints of diabetic foot abscess and wet gangrene of the fifth toe for which he underwent multiple debridement with amputation of the fifth left toe followed by grafting. He gave a history of contact with domestic animals such as street dogs, cats and cattle, with no history of any animal bite.

On admission, his vitals were stable and blood picture showed hemoglobin 11.7 g/dL and raised total leukocyte count of 19.3 × 10^3/μL (neutrophils, 87.7%), with absolute neutrophil count being 16.93 × 10^3/μL. Erythrocyte sedimentation rate was 27 mm/h. The random blood sugar was 194 mg/dL. Serum C-reactive protein was raised (279 mg/L). The patient was taken up for debridement, and intraoperative soft-tissue specimens were sent for microbiological analysis. Gram stain from the specimen showed moderate pus cells with moderate Gram-positive cocci in pairs and clusters and moderate Gram-negative bacilli. The aerobic cultures grew methicillin-resistant *S. aureus* and *Streptococcus dysgalactiae*. The aerobic blood cultures were sterile after 5 days of incubation. The patient was started on empirical antibiotics, tablet

Address for correspondence: Dr. Padmaja Ananth Shenoy, Department of Microbiology, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal - 576 104, Karnataka, India.
E-mail: padmaja.shenoy@manipal.edu

Access this article online

Quick Response Code:

Website: www.jgid.org

DOI: 10.4103/jgid.jgid_345_20

How to cite this article: Shenoy PA, Vishwanath S, Nagaraj RT, Banerjee B, Krishna MS. Diabetic foot infection with *Bacteroides pyogenes*. J Global Infect Dis 2021;13:186-8.

Received: 25 September 2020 **Revised:** 06 October 2020
Accepted: 22 March 2021 **Published:** 07 August 2021

cefepime 500 mg BD, and levofloxacin 500 mg OD. After 24 h of incubation, the anaerobic culture plates (Don Whitley's anaerobic workstation, A35, Yorkshire, UK) showed circular, dome-shaped, mucoid colonies. The colonies were of Gram-negative bacilli, catalase negative, resistant to kanamycin (1000 µg), vancomycin (5 µg), and colistin (10 µg) and were able to grow in the presence of 20% bile suggestive of *Bacteroides* spp. [Figure 1].

Antimicrobial susceptibility test (E test, bioMerieux Inc., Marcy L'Etoile, France) results showed susceptibility to metronidazole (MIC 0.125 µg/mL) clindamycin (MIC 0.016 µg/mL), imipenem (MIC 0.032 µg/mL), meropenem (MIC 0.047 µg/mL), and piperacillin (MIC 0.064 µg/mL). β-lactamase activity (BD BBL Cefinase, Becton Dickinson and Co, Sparks, USA) was not detected. The isolate was identified as *B. pyogenes* by matrix-assisted laser desorption/ ionization–time-of-flight mass spectrometry (VITEK MS, bioMerieux Inc., France) with confidence value of 99.9% with a turnaround time of 48 h. However, VITEK 2 (ANC card, bioMerieux) system identified the same isolate as *Prevotella oralis* with 89% probability. Due to discrepancy in the results by VITEK 2 system and MALDI-TOF, the isolate was subjected to Polymerase chain reaction (PCR).[7] The DNA extraction from culture growth was performed by DNA mini kit (QIAGEN, Hilden, Germany) as per manufacturer's instructions. Primers used were (*gyrB* F) 5'-TGAACACCGAGAAACAAGGC-3' and (*gyrB R*) 5'-TCTCCGGCAATCTCCACTTT-3'. PCR products were resolved by electrophoresis on a 2% agarose gel stained with ethidium bromide. The fragment of approximately 230 bp was considered as presumptive positive for *gyrB* [Figure 2]. Further, the extracted DNA from culture was subjected to 16S rRNA sequencing to confirm the identification. The sequences showed matching results with *B. pyogenes* (Accession no. AB510709.1) with 94% identity.

After 2 days, the patient was taken up for surgical debridement and amputation of the fourth left toe. The repeat intraoperative specimens were sent for anaerobic culture which again grew *B. pyogenes*. His postoperative period was uneventful. The total duration of hospitalization was 10 days, and he was discharged with oral clindamycin 300 mg BD for 5 days. On follow-up visit after 1 month, the wound was healthy. He underwent skin grafting successfully.

Discussion

B. pyogenes is a nonpigmented, saccharolytic, anaerobic Gram-negative bacillus significantly isolated from healthy and diseased subgingival sites of dogs.[3] It may get transmitted to humans through bite, however, the pathogenesis is not clearly described in literature.[3] *B. pyogenes* has shown 100% similarity to *Bacteroides suis* and *Bacteroides tectus* with respect to 16S rRNA genes. Although *B. suis* and *B. tectus* are considered as heterotypic synonyms of *B. pyogenes*, their biochemical properties may vary [Table 1].[7]

In humans, *B. pyogenes* is known to cause soft-tissue infections and bloodstream infections following cat or dog bite, which tend to be polymicrobial in nature constituting their oral microbiota.[4,6]

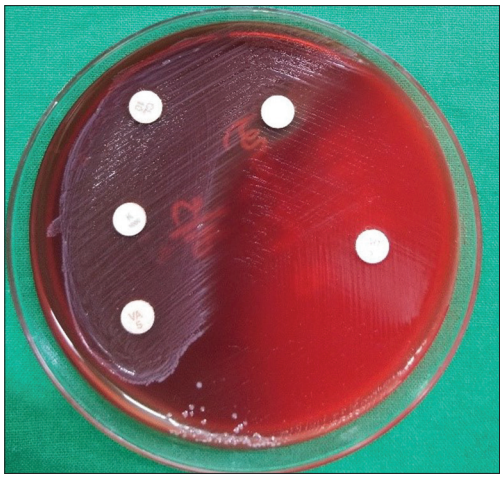

Figure 1: Five percent sheep blood agar plate showing *Bacteroides pyogenes* growth

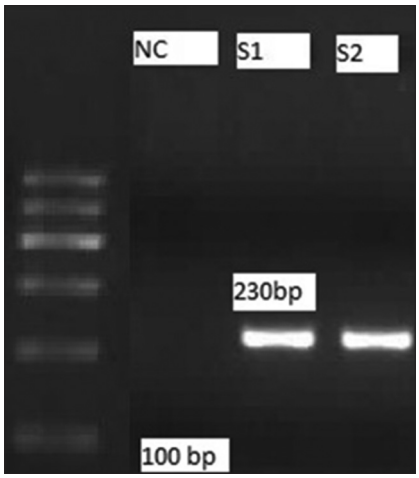

Figure 2: Agarose gel showing polymerase chain reaction amplification products of *Bacteroides pyo*genes-*gyrB* gene

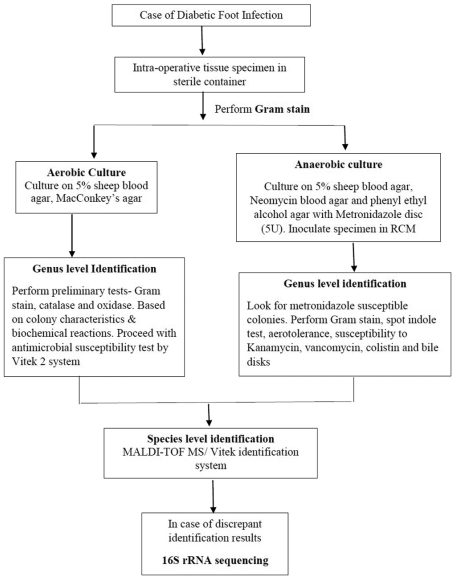

Figure 3: Algorithm for bacterial identification of microbial cultures in diabetic foot infections

Table 1: Microbiological characteristics of *Bacteroides pyogenes, Bacteroides suis,* and *Bacteroides tectus*

Stain and biochemical properties	Result from Sakamoto *et al*., 2010[7]			*B. pyogenes* (Madsen and *Justesen*, 2011)[4]	Present study results
	B. pyogenes	*B. suis*	*B. tectus*		
Gram reaction	Gram-negative	Gram-negative	Gram-negative	Gram-negative	Gram-negative
Catalase	Negative	Negative	Negative	Negative	Negative
Indole test	Negative	Negative	Negative	Negative	Negative
Urease	Negative	Negative	Negative	-	Negative
β-Glucosidase	Positive	Positive	Negative	Positive	Positive
Acid production from					
Glucose	Positive	Positive	Positive	Positive	Positive
Cellobiose	Negative	Positive	Negative	Negative	Negative
Mannose	Positive	Negative	Positive	Positive	Positive
Sucrose	Positive	Positive	Negative	Negative	Positive
Sorbitol	Negative	Positive	Negative	Negative	Negative
Mannitol	Negative	Negative	Negative	-	Negative
β-Lactamase production	-	-	-	Negative	Negative

The biochemicals of the present study were interpreted from VITEK card (ANC card, bioMerieux).

Furthermore, there are reported cases of prosthetic joint infections and bacteremia without any history of animal bite.[5,8] The presence of open wound and intimate contact with pet animals may increase the risk with *B. pyogenes* infection.[5] In the present case, the patient was diabetic with no history of any animal bite although contact with street animals was present. In all the reported human infections associated with *B. pyogenes,* either lower/upper extremities are commonly affected.[5,6,8] The isolation of *B. pyogenes* in routine anaerobic cultures is infrequent, however, *B. pyogenes* can be differentiated from *Bacteroides fragilis* with the absence of detectable β-lactamase activity in former.[4,9] Furthermore, it is likely that *B. pyogenes* may be misidentified as *B. fragilis* in laboratories not adopting automated microbial identification system or not performing extensive biochemical tests for identification. As the antimicrobial resistance pattern may vary among diverse species of genus *Bacteroides*, the accurate species level identification is need of the hour. *B. pyogenes* is known to be susceptible to most of the anti-anaerobic drugs including penicillin group.[4,8] Satisfactory clinical improvement to therapy was noted in this case.

Infected diabetic foot ulcers comprise mixed population of bacteria. The presence of various virulence factors may contribute to chronicity of ulcers leading to delay in clinical outcome, thus making it necessity to identify the etiological agents which may predispose to chronic infections. The proper collection of specimen, involvement of transport media, and following step-wise microbiological diagnostic algorithm [Figure 3] play a significant role in clinical management.[10]

Mass spectrometry was successful in identification of *B. pyogenes* in this case, and 16S rRNA sequencing would be a useful tool for confirmatory identification of clinically significant *Bacteroides* sp.[7]

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given his consent for his images and other clinical information to be reported in the journal. The patient understands that his name and initial will not be published, and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

Research quality and ethics statement

The authors followed applicable EQUATOR Network ("http://www.equator-network.org/) guidelines, notably the CARE guideline, during the conduct of this report.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Goh TC, Bajuri MY, Nadarajah SC, Abdul Rashid AH, Baharuddin S, Zamri KS. Clinical and bacteriological profile of diabetic foot infections in a tertiary care. J Foot Ankle Res 2020;13:36.
2. Wexler HM. *Bacteroides*: The good, the bad, and the nitty-gritty. Clin Microbiol Rev 2007;20:593-621.
3. Forsblom B, Love DN, Sarkiala-Kessel E, Jousimies-Somer H. Characterization of anaerobic, gram-negative, nonpigmented, saccharolytic rods from subgingival sites in dogs. Clin Infect Dis 1997;25 Suppl 2:S100-6.
4. Madsen IR, Justesen US. Bacteremia with *Bacteroides pyogenes* after a cat bite. J Clin Microbiol 2011;49:3092-3.
5. Gual-de-Torrella A, Suárez-Barrenechea AI, Del Toro MD. *Peptostreptococcus canis* and *Bacteroides pyogenes* prosthetic joint infection. Enferm Infecc Microbiol Clin 2019;37:347-8.
6. Lau JS, Korman TM, Yeung A, Streitberg R, Francis MJ, Graham M. *Bacteroides pyogenes* causing serious human wound infection from animal bites. Anaerobe 2016;42:172-5.
7. Sakamoto M, Suzuki N, Benno Y. hsp60 and 16S rRNA gene sequence relationships among species of the genus *Bacteroides* with the finding that *Bacteroides suis* and *Bacteroides tectus* are heterotypic synonyms of *Bacteroides pyogenes*. Int J Syst Evol Microbiol 2010;60:2984-90.
8. Park JE, Park SY, Song DJ, Huh HJ, Ki CS, Peck KR, *et al*. A case of *Bacteroides pyogenes* bacteremia secondary to liver abscess. Anaerobe 2016;42:78-80.
9. Jousimies-Somer H, Summanen P, Citron DM, Baron EJ, Wexler HM, Finegold SM (2002). Wadsworth-KTL Anaerobic Bacteriology Manual. 6th ed. California: Star Publishing Company; 2002.
10. Sadeghpour Heravi F, Zakrzewski M, Vickery K, G Armstrong D, Hu H. Bacterial diversity of diabetic foot ulcers: Current status and future prospectives. J Clin Med 2019;8:1935.