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The role of anaerobes in diabetic foot infections

Patrick G.P. Charles [a, b, c, *], Ilker Uçkay [a, d, e], Benjamin Kressmann [a, d], Stéphane Emonet [a, f], Benjamin A. Lipsky [a, g]

[a] Infectious Diseases Service, Geneva University Hospitals and Faculty of Medicine, Geneva, Switzerland
[b] Department of Infectious Diseases, Austin Health, Heidelberg, Australia
[c] Department of Medicine, University of Melbourne, Parkville, Australia
[d] Orthopaedic Surgery Service, Geneva University Hospitals and Faculty of Medicine, Geneva, Switzerland
[e] Infection Control Program, Geneva University Hospitals and Faculty of Medicine, Geneva, Switzerland
[f] Laboratory of Bacteriology, Geneva University Hospitals, Geneva, Switzerland
[g] Division of Medical Sciences (Infectious Diseases), University of Oxford, Oxford, UK

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ABSTRACT

Diabetic foot infections (DFI) are a common cause of morbidity and, on occasion, even mortality. Infection can be either mono- or polymicrobial, with a wide variety of potential pathogens. Anaerobes may be involved, particularly in wounds that are deeper or more chronic, and are more frequently identified when using modern molecular techniques, such as 16s PCR and pyrosequencing. It remains unclear whether the presence of anaerobes in DFI leads to more severe manifestations, or if these organisms are largely colonizers associated with the presence of greater degrees of tissue ischemia and necrosis. Commonly used empiric antibiotic therapy for diabetic foot infections is generally broad-spectrum and usually has activity against the most frequently identified anaerobes, such as *Peptostreptococcus* and *Bacteroides* species. Adequate surgical debridement and, when needed, foot revascularization may be at least as important as the choice of antibiotic to achieve a successful treatment outcome.

1. Introduction

Infection in the feet of people with diabetes continues to be a major problem throughout the world. For those with diabetes, the lifetime risk of developing a diabetic foot infection (DFI) is currently estimated to be about 15–25% [1]. With the rising prevalence of diabetes mellitus worldwide, the longer lifespan of these patients and the high likelihood of recurrence of DFIs, the number of cases is likely to substantially increase. DFI are associated with substantial morbidity, poor quality of life, high risk of lower extremity amputations, need for hospitalization and even deaths [2,3]. Most acute infections in patients not recently treated with antibiotic therapy are monomicrobial and, at least in Western countries, caused predominantly by aerobic gram-positive cocci (especially *Staphylococcus aureus*). Infections that are chronic, or have been previously treated with antimicrobials, often are polymicrobial, typically with the addition of aerobic or facultative anaerobic gram-negative bacilli [3].

Obligate anaerobic bacteria, although undoubtedly long-noted in some DFI, were first discussed as potentially important pathogens in diabetic foot wounds in 1976 [4]. Almost two decades later, in the earliest published review of the role of anaerobes in soft tissue and bone DFIs, Gerding noted that the rates of isolation of anaerobes in DFIs varied greatly, but was directly associated with deeper, more severe infections and employing proper sampling, transport and culture techniques [5]. Despite many studies of DFI in the subsequent 20 years, the true frequency of anaerobic pathogens in DFI remains unclear, largely related to a lack of standardization of bacterial culture methods among studies, especially concerning the type of sample taken for analysis (e.g., surface swab, needle aspirate or biopsy), the method and efficiency of transportation of samples to the microbiology laboratory and how they are processed there [4,5]. Furthermore, as noted by Gerding and still true today, the clinical significance of isolated anaerobes remains in doubt [5]. The rate of anaerobic infection does appear to be highest in ischemic or necrotic wounds, where the impaired blood supply and low redox potential may facilitate their proliferation.

* Corresponding author. Department of Infectious Diseases, Austin Health, PO Box 5555, Heidelberg, VIC 3084, Australia.
E-mail address: patrick.charles@austin.org.au (P.G.P. Charles).

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Table 1
Clinical severity of diabetic foot infections.

IDSA infection severity	PEDIS grade	Symptoms or signs
Uninfected	1	No symptoms or signs of infection
Mild	2	Local infection[a] of skin or subcutaneous tissues. Any erythema extends ≤2 cm from the rim of an ulcer. Other causes of inflammation excluded.[b]
Moderate	3	Local erythema extending >2 cm from ulcer rim or infection that extends deeper than the skin and subcutaneous tissues, but no systemic inflammatory response syndrome
Severe	4	Local infection plus the presence of at least two features of systemic inflammatory response syndrome: • Temperature >38 °C or <36 °C • Heart rate >90 beats per minute • Respiratory rate >20 breaths per minute or $PaCO_2$ < 32 mmHg • White blood cell count >12,000 or <4000 cells/μ>L or ≥10% immature or band forms).

[a] Evidence of infection is defined by the presence of at least two of the following:
- Local swelling or induration
- Erythema
- Local pain or tenderness
- Local warmth
- Purulent discharge.

[b] Other potential causes that should be considered and excluded include trauma, gout, acute Charcot neuro-osteoarthropathy, fracture, thrombosis and venous stasis.

2. Pathogenesis and clinical aspects

Most DFI begin in a foot wound, which usually follows non-perceived trauma secondary to the loss of protective sensation in a patient with peripheral polyneuropathy. Additional contributing factors in DFI include impairment in arterial blood supply, metabolic effects of chronic hyperglycemia, and poorly understood types of immune dysfunction [6]. When there is a break in the protective skin envelope, bacteria will contaminate then colonize the exposed subcutaneous tissues. These microbes can originate from the environment, the neighboring skin or other endogenous sources, including the gastrointestinal tract. Bacterial growth is enhanced by the presence of tissue ischemia (resulting in hypoxia) or necrosis [7]. This situation is ideal for bacterial infection, defined as proliferation of pathogenic microbes with resultant host response and tissue damage. Infection in the wound can progress contiguously to involve deeper tissues, and in some instances to become systemic.

At the time of initial presentation, approximately half of all diabetic foot lesions are clinically uninfected (PEDIS Grade 1; see Table 1), i.e., they show no local signs of inflammation and there is no systemic inflammatory response [3,8,9]. When the wound becomes infected the severity is usually initially mild, involving only the skin and subcutaneous tissue, with limited cellulitis. As noted above, these PEDIS grade 2 DFI are typically caused by aerobic gram-positive cocci, most commonly *S. aureus*. If the DFI progresses to a moderate (PEDIS grade 3, becoming more extensive or involving deeper tissues) or severe (PEDIS grade 4, associated with the systemic inflammatory response syndrome) polymicrobial infections, with aerobic gram-negative bacilli and anaerobes joining the gram-positive cocci, are common. Anaerobes are predominantly seen in DFI with ulcers that are deeper, more chronic, associated with ischemia, necrosis, gangrene or foul odor [3,6,10].

3. Microbiology of DFI

The type of sample sent to the microbiology laboratory, and the methods used to transport it there for processing, both have a major bearing on the ability to identify anaerobic bacteria in DFI. Superficial swabs are a poor method for sampling open wounds, as they lack both specificity and sensitivity compared to tissue specimens [3,11,12]. Before collecting any sample, including tissue specimens, it is important to first clean and debride the wound prior to reduce the rate of false positive cultures by avoiding collecting colonizing flora [3]. In patients with suspected underlying osteomyelitis, cultures of aseptically collected bone have generally provided more reliable results than either ulcer swab or deep tissue needle biopsy [13,14].

Culturing anaerobes has always been challenging for routine clinical microbiology laboratories. One major reason for this is the difficulty in constantly maintaining a strict anaerobic atmosphere. A key method to enhance recovery of anaerobes is to minimize the time from specimen collection to anaerobic incubation. Further, the laboratory should have specific anaerobic compartments dedicated to each day of the week, to avoid re-opening of the incubator when inserting new plates. Using non-selective CDC anaerobe 5% blood sheep agar favors the growth of anaerobes. However, as diabetic foot infection is often polymicrobial, organisms such as members of the Enterobacteriaceae, *Streptococcus anginosus* group and other aerobic bacteria with the ability to grow in anaerobic conditions may potentially outcompete the strict anaerobes, and lead to them being missed. The use of selective media, such as brain heart infusion (BHI) agar containing gentamicin and vancomycin, will enhance the ability to grow gram-negative anaerobes such as *Bacteroides fragilis* in polymicrobial specimen, but at the expense of losing many gram-positive anaerobes.

Despite maximal clinician and laboratory efforts, cultures miss many obligate anaerobes and other fastidious bacteria. With the rapid improvement of molecular sequencing techniques, we are entering a new era of microbiology. Newer techniques, such as 16s polymerase chain reaction (PCR) and pyrosequencing, typically identify a higher number of bacteria, including anaerobes, than standard culture-based techniques. The microbiome identified using such PCR techniques has been termed the “bioburden”, with concerns that a higher bioburden may be associated with delays in healing as a result of higher microbial load, greater microbial diversity and presence of more pathogenic bacterial species [10,15]. Metagenomic studies have revealed that there is interplay among bacterial communities in specific environments, such as wounds, to produce specific clinical “syndromes” [16–18]. Certainly, various neurological, vascular and mechanical factors are known to affect the appearance and outcome of wounds. In the future, studying the wound microbiome may help answer questions such as why certain DFI patients present with more acute cellulitis, whereas others develop deep and chronic ulcers, and some resolve quickly. By way of example, a recent study has shown that the deeper and more chronic a diabetic foot ulcer, the more prevalent are anaerobic and gram-negative bacteria, and the less prevalent are staphylococcal species, although a direct cause–effect relationship for these findings was not established [10].

Currently, the clinical significance of bacteria identified only by