



Bordetella trematum in Diabetic Foot Infection, a Serious Cause to Explore

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Abstract

Reporting a case of *Bordetella trematum* in a diabetic foot ulcer.

Bordetella trematum is an infrequent gram-negative coccobacillus, typically related to tissue infections.

Monitoring the appearance of new cases of *B. trematum* is essential, since it can be an emerging microorganism. Isolating and defining the clinical relevance of unusual bacteria yields a more accurate perspective in the development of new diagnostic tools and allows for assessment of proper antimicrobial therapy.

Keywords: Diabetic foot; *Bordetella trematum*; Infection; Gram-negative coccobacilli

Introduction

Bordetella trematum is a rare gram-negative coccobacillus [1] that has been mainly associated with tissue infection, isolated from ear, leg, arm and ankle wound infections or from diabetic foot ulcers. Specific information about the source and susceptibilities to this coccobacillus are still very scarce given the rare frequency of this isolate [2].

Case Presentation

A 71-year-old male, known to have type-1 diabetes for 20 years, on insulin, suffering from multiple organ micro- and macro-angiopathies, namely retinopathy and severe occlusive Peripheral Arterial Disease (PAD) was admitted for split thickness skin graft to his right foot dorsum.

His problem started 6 months prior to presentation when he developed wet gangrene of his second, then third right foot toes. Upon consultation and investigations with a vascular surgeon the followings were observed:

Arterial Doppler showed multi-focal atherosclerosis and calcifications more prominent at the adductor hiatus and below knee, associated with multi-focal stenosis. The distal aspect of the anterior tibial and dorsalis pedis were patent with hyperthermic monophasic flow, in keeping with distal infection.

Computed tomography-angiography revealed total occlusion of the anterior and posterior tibial arteries. The peroneal artery was patent in its lower two thirds showing severe atherosclerosis with occlusion of its proximal one third.

Pre-operative arteriography showed an occluded right popliteal artery with distal peroneal artery filling. A right femoro-peroneal artery autologous bypass surgery was performed.

Following surgery however, the fourth and fifth toes developed wet gangrene and a severe infection that spread in the dorsum of the foot with swelling, cellulitis and large patchy zones of necrosis reaching the lateral malleolus (Figure 1). The distal surgical arterial bypass wound in the leg got infected and became fully dehiscent (Figure 2). The patient general condition worsened rapidly and a knee level amputation proximal to the dehiscent bypass wound was suggested and discussed with the patient who refused to undergo this amputation. Therefore, an amputation of the 4 lateral gangrenous toes along with their respective metatarsal heads was performed to preserve the intact big toe followed by a radical dorsal foot fasciectomy after excision of all devitalized dorsal foot soft tissues (Figure 3). Further, a Negative Pressure Wound Therapy (NPWT) was applied to remove

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Figure 1: Multiple ulcers on the right foot dorsum of the diabetic patient, with adjacent redness and swelling.



Figure 4: Wound ready for skin graft.



Figure 2: Site of previously infected arterial bypass surgery.



Figure 5: Wounds post-split thickness skin graft surgery.



Figure 3: Anterior tibial ulcer after debridement.



Figure 6: Wound healing of the lesions six days post-split thickness skin graft surgery.

excess exudates and promote healing. The patient was discharged on Ciprofloxacin 500 mg twice per day for two weeks. Ten weeks later, the large wound granulated fairly well with no clinical signs of infection (Figure 4) and the patient was admitted to perform a Split Thickness Skin Graft (STSG) (Figure 5). Prior to STSG, several swabs were collected from the wound and sent to the hospital's microbiology laboratory for microbiological analysis.

Microscopic examination of the sample after a Gram stain (RAL diagnostics) showed few neutrophils and Gram-positive cocci. Sample swabs were Seeded on Blood Agar base (SBA) Bio-Rad[®] supplemented with 5% sheep blood, Colistin-Nalidixic Acid Agar (CNA) Bio-Rad[®], Chocolate Agar (CA) Mast Group[®], and MacConkey agar (MC) Bio-Rad[®]. The sample was also cultured for enrichment in the Thioglycolate Broth (TB) Mast Group[®] for 24h/35°C. After incubation, growth of Gram-negative bacilli was seen on SBA, CA and MC agar while Gram-positive cocci grew on SBA and CA. TB enriched sample was inoculated on CNA and MC for an additional

24 h. Gram-negative bacilli grew on MC and Gram-positive cocci on CNA. Catalase-positive and coagulase-positive (Pastorex[™] Staph-Plus) tests added to the ApiStaph, bioMérieux[®] confirmed the Gram-positive cocci as *Staphylococcus aureus*.

Biochemical identification of the oxydase-negative (Mast Group[®]) and Gram-negative bacilli colonies using Api 20 NE[®] (bioMérieux) showed 72.3% *Achromobacter denitrificans* and 16.9% *Bordetella bronchiseptica*. Colonies were also sent for identification by Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF Vitek-MS, BioMérieux) and the result came as *Bordetella trematum*.

Susceptibility testing of *B. trematum* colonies by disk diffusion on Mueller Hinton Agar (MHA) (Bio-Rad[®]) using antimicrobial disks (Bio-Rad[®]) showed (Table 1) susceptibility to Ampicillin (AMP), Amoxicillin-Clavulanic Acid (AMC), Piperacillin-Tazobactam (TZP), Cefazidime (CAZ), Imipenem (IPM), Meropenem (MEM), Gentamicin (GEN), Nalidixic Acid (NAL), Trimethoprim-Sulfamethoxazole (SXT), while it showed resistance to Cefuroxime (CXM), Levofloxacin (LVX) and Ciprofloxacin (CIP). In addition, antimicrobial susceptibility testing for *B. trematum* was performed to obtain minimal inhibitory concentrations using the MIC Test strip by Liofilchem[®] *in vitro* (in µg/mL; Amoxicillin 0.75; Cefazidime 3;

Amoxicillin-clavulanate 0.38; Ertapenem 0.016; Tigecycline 0.75; Imipenem 0.25) (Table 2).

Following the debridement and prior to culture and susceptibility results report, the patient was prescribed Ciprofloxacin and his condition improved (Figure 6). He was discharged 6 days after the graft.

Discussion

Bordetella trematum originated from the word “trema” referring to pierced or infiltrated [1]; it was first described by Vandamme et al. They performed a comprehensive phenotypic and genotypic analysis of 10 atypical or unclassified *Bordetella* strains and found that these strains belonged to a new species of *Bordetella*. All of these had been isolated from ear infections or leg, arm and ankle wounds [3] in which this bacterium can be located in open wounds or bare parts of the body and do not colonize the respiratory tract unlike other *Bordetella* species [1]. Details regarding its reservoir, life cycle and pathogenesis as well as virulence remain largely unknown [4].

When *B. trematum* was previously prescribed, it presented the following phenotypic characteristics: Grown on MacConkey agar, non-glucose metabolizer, motile, with variable nitrate reduction, catalase- and citrate-positive, urease-, oxidase- and lysine decarboxylase-negative [1]. In this case, MALDI-TOF MS analysis was the only efficient methodology to accurately identify the organism.

A misidentification using biochemical method Api 20 NE[®] mislead our diagnosis, since the identified microorganisms were *Achromobacter denitrificans*/*Bordetella bronchiseptica*, which did not clinically justify the skin lesion nor the medical presentation. Such discrepancies were previously described and attributed to the test variability of nitrate reduction, and the variation of oxidase test reagent from the one used by other researchers [3]. However, other misidentification in the use of Api 20 NE[®] were also attributed to the fact that *B. trematum* was missing in the manufacturer identification database [5] used in practice by microbiology laboratories. In other reports the identification of the microorganism was validated by 16S rRNA gene sequencing [3,4,6-8]. The Clinical Laboratory Standard Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing did not standardize any methodology that performs an antimicrobial susceptibility test, specifically for *B. trematum* [2].

Conclusion

Bordetella trematum is a rare gram-negative rod [1] isolated from different clinical specimen. A recent review of literature noted eleven cases of human *B. trematum* infections [2,9,10], the majority of which were related to chronic skin ulcers with polymicrobial infections [7].

The combination of antibiotic therapy and surgical debridement plays a key role in preventing systemic infections [8]. The advent of MALDI-TOF MS allows for a more accurate and rapid identification and could elucidate the ‘real’ epidemiology of *B. trematum* infections in particular and rare species in general. An increased awareness is essential for estimating the importance of *B. trematum* as a possible emerging pathogen [4].

Author Contributions

JH collected data. GK analyzed, interpreted the data and put the diagnosis with SH.

SH wrote the manuscript and cover letter. GK revised the manuscript. All authors have reviewed and approved the manuscript.

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