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CASE REPORT

Post-traumatic infection of the lower limb caused by rare Enterobacteriaceae and Mucorales in a young healthy male☆

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Summary *Enterobacter amnigenus* and *Leclercia adecarboxylata* are Gram-negative aerobic bacilli of the family Enterobacteriaceae that have been isolated from water and, rarely, from various clinical specimens. Absidia is a filamentous fungus of the class Zygomycetes that is ubiquitous in nature and can cause infection, primarily in immunocompromised hosts. Here, we describe an infection of the left lower limb caused by *E. amnigenus* and *L. adecarboxylata* with subsequent isolation of *Absidia* spp. in a patient with multiple traumatic injuries after a major motor vehicle accident. The severity of the clinical picture made amputation necessary, despite aggressive anti-infective therapy with both antibacterial and antifungal agents. Prompt diagnosis and management are mandatory in order to minimize morbidity and even mortality, and reduce the social and economic cost.

Introduction

Enterobacter amnigenus and *Leclercia adecarboxylata* are motile, Gram-negative aerobic bacilli (GNAB) of the family Enterobacteriaceae. They are ubiquitous in nature and have been isolated from water and soil and also from various clinical specimens, including blood, feces, sputum and wounds.[1,2] *E. amnigenus* has been grown in samples from a few patients with sepsis[3] and other infections.[4] *L. adecarboxylata* has been found to cause infection both in immunocompromised hosts (bacteremia) and in otherwise

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healthy adults (as part of mixed microbial growth in wound infections of the lower limbs).[5] Both these GNAB are susceptible to all the antimicrobial agents that are generally active against Enterobacteriaceae (most beta-lactams, aminoglycosides, fluoroquinolones, antifolates, chloramphenicol, nitrofurantoin), except for fosfomycin.[6,7]

Absidia species — like other species of the order Mucorales (i.e. Mucor and Rhizopus) — are filamentous fungi of the class Zygomycetes that are ubiquitous in nature, and have been isolated from humid soil, plant debris and air.[8] *Absidia* spp. are common laboratory contaminants and only *Absidia corymbifera* is considered pathogenic for humans. *A. corymbifera*, for which reclassification as *Mycocladus corymbifer* has been recently proposed,[9] very rarely causes infection in the immunocompetent host, whereas its growth in culture from clinical samples of immunosuppressed and diabetic subjects should be regarded as potentially significant.[10] Traditionally, amphotericin B has been the only effective antifungal drug against Zygomycetes, which are resistant to flucytosine, caspofungin, fluconazole and voriconazole;[11] however, the new triazole agent posaconazole has a good activity.[12]

Here, we describe a mixed infection of the left lower limb caused by *E. amnigenus* and *L. adecarboxylata* with subsequent isolation of *Absidia* spp. in a patient with severe polytrauma.

Case report

A 37-year-old Caucasian male — body weight approximately 65 kg — was admitted to our Emergency Department (ED) after a major motor vehicle accident. He had the following multiple injuries: Gustillo—Anderson type II open fracture of the left distal femur; type IIIB open fracture of the left distal tibia with bone loss; and mangled left foot with multiple fractures and extensive soft-tissue involvement. The patient was rescued on the road, so no farm contamination was expected. In the ED, he was treated with irrigation and débridement (I&D). In an attempt to save the left lower limb, open reduction and internal fixation (ORIF) of the foot and distal tibia fractures was performed, together with external fixation of the femur.

Intravenous empiric antibiotic therapy was promptly initiated according to hospital protocol: teicoplanin 400 mg/day (after a loading dose of 800 mg), tobramicin 300 mg/day and metronidazole 500 mg four times per day. Tetanus prophylaxis was not supplied as the patient had received a booster dose one year before.

Angiography and a microsurgical referral were obtained; it was decided to wait for necrosis delimitation and a course of hyperbaric oxygen therapy was initiated.

After 14 days, the clinical situation showed no possibility of limb salvage and a below-knee amputation was performed. Multiple surgical samples of the wound (swabs, débrided tissue) were collected for microbiological cultures, which grew two enterobacterial strains after isolation on Columbia blood agar plates incubated at 37 °C for 48 hours. Their identification as *E. amnigenus* and *L. adecarboxylata* was performed by standard techniques, that is, biochemical identification and susceptibility testing using a commercial automated system (Vitek 2, bioMérieux, France). Therefore, targeted antibiotic therapy with intravenous piperacillin/tazobactam 4.5 g three times per day was undertaken in place of the previous treatment.

Figure 1 Stump of the left lower limb at the time of the second amputation surgery. The presence of grayish plaques with necrotic edges in two distinct areas (delimited by the black line) suggested Absidia etiology, thereafter confirmed by direct microscopy.

After seven days, a second surgery, involving I&D plus re-amputation on a more proximal level (but always below knee), was needed to control a recalcitrant infection of the stump; grayish plaques with necrotic edges had appeared and rapidly increased in size (Figure 1). This type of skin lesion is suggestive of Absidia infection.[10] New intra-operative samples of the wound were collected. The culture, after inoculation onto Sabouraud dextrose agar, grew a fungus that was consistent with *Absidia* spp. on the basis of both colony morphology (woolly grayish surface) and microscopic features (large non-septate filamentous hyphae, branched sporangiophores with flask-shaped apophysis just below the columella).[10]

Antifungal therapy with liposomal amphotericin B 3 mg/kg (200 mg) per day was prescribed at that time and continued for 15 days. Thereafter, final ORIF of the distal femur was performed and control of stump infection was finally achieved.

Eighteen months after the final surgery, no signs of infection were present at both stump and femur level.

Discussion

In the medical literature, there are scant citations on human infections associated with particular Enterobacteriaceae and filamentous fungi. *E. amnigenus*, previously called *Enterobacter* H3, was reported to have caused sepsis only in two patients, after blood transfusion[3] and heart transplantation.[13] Other site infections (cholecystitis, cystitis, lymphadenitis, osteomyelitis) have also been reported to be caused by this microorganism.[4] During the past decade, *L. adecarboxylata* has most often been isolated from clinical specimens as part of mixed flora in trauma- or diabetes-associated wound infections of the lower limbs[5,14] and in immunosuppressed patients with sepsis and abdominal infections.[5,15] It is possible that the development of commercially available

computer-based identification systems and newer and more accurate methods (DNA—DNA hybridization, specific bacteriophage) has contributed to the increasingly frequent identification of *L. adecarboxylata* after isolation from clinical specimens, as *L. adecarboxylata* can be easily confused with *Escherichia coli* in the standard laboratory.

Infections with *Absidia* spp. usually occur in immunocompromised hosts, such as patients with acute leukemia. They manifest with respiratory, gastrointestinal, cerebral or sinusal symptoms, and are often fatal, even if aggressive antifungal treatment is used.[16] Moreover, skin and soft-tissue infections have been described in HIV-infected subjects[17] and in burn patients, where their occurrence is facilitated by moist and humid conditions, as in the case of occlusive bandages.[18]

We described a mixed infection of the left lower limb caused by *E. amnigenus* and *L. adecarboxylata* with subsequent isolation of *Absidia* spp. in a patient with severe traumatic injuries. We cannot exclude that the filamentous fungus was present before the first surgical procedure, as infections with *Absidia* spp. can be caused even by traumatic implantation through skin—ground contact in previously healthy subjects. Such cases can be fatal[19] or require aggressive surgical treatment (drainage, débridement, resection), including amputation, despite appropriate antifungal therapy with amphotericin B or posaconazole.[20,21] This emphasizes the need for early diagnosis and, subsequently, prolonged treatment in order to obtain a successful outcome.[22] Otherwise, it is also possible that our patient's Absidia strain was transmitted nosocomially during surgery or hospital stay, as previously described in kidney, liver and bone marrow transplantation subjects.[23—25] Our patient had no pre-existing evidence of immune suppression, but acute post-traumatic shock could lead to a low-to-moderate depression of immune function, which, in turn, favors the appearance of a severe Absidia infection of the lower limb. Moreover, a typical risk factor for mycetic and mold contamination, that is, a recently prescribed broad-spectrum antibiotic therapy, must be considered for our patient.

As far as the susceptibility patterns of our enterobacterial isolates are concerned, *E. amnigenus* was fully susceptible to aminoglycosides, aztreonam, carbapenems, third- and forth-generation cephalosporins, cotrimoxazole, fluoroquinolones and ureidopenicillins, and resistant to aminopenicillins and first-generation cephalosporins (cefazolin) only, as has sometimes been reported.[6] On the other hand, *L. adecarboxylata* was fully susceptible to all tested antimicrobial agents (beta-lactams including aminopenicillins and first-generation cephalosporins, aminoglycosides, fluoroquinolones, cotrimoxazole), which are generally effective against *E. coli* as well.[7] Despite the use of antibiotics that were active in vitro against both Enterobacteriaceae (first tobramycin, then piperacillin/tazobactam) and although therapy with full-dose liposomal amphotericin B was started when the coexistence of Absidia emerged, the severe clinical picture continued and it was not possible to rescue the limb from amputation.

This case report on severe post-traumatic infection of a lower limb emphasizes the importance of both prompt wound débridement and systemic antibacterial therapy. Additionally, fundamental microbiological work-up for bacterial and/or fungal identification and consequent targeted antimicrobial therapy are essential in order to minimize morbidity and even mortality, and reduce the need for major surgical procedures.

Conflict of interest: No conflict of interest to declare.

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Ethical approval was not required for this study.

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