CASE REPORT

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A case report of a multi-drug resistant bacterial infection in a diabetic patient treated in northeast Brazil

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Diabetes mellitus is one of the most critical health conditions around the world, not only in terms of the number of affected people, disability, and premature mortality, but also in regards to the health care costs involved in controlling and treating its complications. Among the most constant ailments the diabetic patient suffers is the diabetic foot, defined as any infection, ulceration, and/or necrosis of deep tissues associated with neurological abnormalities and various degrees of peripheral vascular disease of the lower limbs. Diabetic foot ulcerations have become a major and increasing public health concern and its associated morbidities, impairment of the patients' quality of life, and the implied costs for management have attracted the attention of numerous health care providers. In this case report, the authors review a unique presentation of a polymicrobial infection of a multi-drug resistant character species formed by oxacillin-resistant *Staphylococcus aureus*, *Acinetobacter baumannii* and *Acinetobacter lwoffii*.

Keywords: *diabetic foot*; *ulcer*; *neuropathy*; *resistance*; *polymicrobial infection*

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According to the American Diabetes Association, diabetes mellitus (DM) is defined as a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. This condition of chronic hyperglycemia is associated, after long periods, with injury and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (1).

The diabetic population is currently increasing worldwide, especially in developing countries like Brazil (2). In 1985, there were approximately 30 million adults suffering from diabetes in the world. In 1995, this figure amounted to 135 million, while in 2002 the number of diabetics was 173 million and is expected to reach a total of 300 million in 2025. About two-thirds of diabetics live in developing countries where the epidemic is most intense with an increasing proportion in younger age groups (2).

According to a study conducted between 1986 and 1988 in a population ranging from 30 to 69 years of age, Brazil's diabetes prevalence rate was 7.6% (3). A more recent study conducted in Ribeirão Preto, São Paulo, showed a prevalence of 12.1% DM in the same age group (4). It is estimated that in 2005, the number of diabetics in Brazil amounted to an alarming figure of 8 million people (1). In the state of Rio Grande do Norte, the prevalence of diabetes accounts for 9.4% and the hospitalization rate due to complications from the disease is equivalent to 2.7% (5).

It is well known that diabetic patients have a high risk of developing complications including and not limited to retinopathy, nephropathy, and neuropathy. Among these complications, peripheral neuropathy is directly associated with the onset of ulcers in the lower limbs (6). Considering the various chronic complications associated with diabetes, the diabetic foot is particularly considered as the main cause of hospital admissions for a significant portion of diabetic patients, and is also a major cause of prolonged hospitalization among these patients (6). World population data indicate that about 82,000 people have diabetes-related amputations of feet and lower extremities each year (6). It is also estimated that 14–20% of diabetic patients with foot ulcers undergo an amputation, while 85% of amputations are preceded

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by ulcers (7). The ulcers result from multiple pathophysiological mechanisms, and in diabetic patients, this is mainly due to a complication of such a critical illness (8).

Neuropathy acts in a permissive way, and is present in about 90% of patients with injury, demystifying the perceived notion that the diabetic foot is mainly a vascular complication. However, the incidence of distal arterial lesions is higher in DM patients than in people without diabetes (8). Generally, peripheral vascular disease (PVD) is not an independent risk factor for these ulcers, but when associated with neuropathy it is a major cause of non-traumatic amputations. Critical limb ischemia hinders the healing process given the body's inability to provide nutrients and oxygen to the wound bed. Infection is also more common by harming the immune system and preventing antibiotic's effectiveness due to ischemia (8). The association between DM and PVD can trigger the onset of a neuro-ischemic ulcer and its prevalence is 34%. Ulcers caused by other factors not associated with DM correspond to 1%. Each complication also increases the host's susceptibility for developing a different lesion (8).

The Wagner classification system is a widely accepted stratification of diabetic foot ulcers, which, for the most part, are infected with polymicrobial flora consisting of aerobic, gram-negative, gram-positive, and anaerobic bacteria (9). It is generally accepted that the majority of mild to moderate infections are caused by gram-positive bacteria, while severe infections and/or chronic diseases are often polymicrobial, involving gram-negative and anaerobic bacteria, which are responsible for 20–60% of the hospitalizations (10, 11).

The emergence and spread of microorganisms with multi-drug resistance is currently considered as a major public health concern, given its growing incidence in both hospitals and communities. In a patient exposed to antibiotics, resistant organisms may emerge by natural selection through the expansion of subpopulations generated spontaneously (12). The multi-drug resistant organisms of greater importance in the hospital environment include oxacillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus* species (VRE), multi-resistant *Streptococcus pneumoniae*, gram-negative organisms, and multi-resistant gram negative bacteria including *Pseudomonas* species, *Acinetobacter* species, *Klebsiella pneumoniae*, *Enterobacter* species and other organisms (13). Given this resistance's panorama, it is necessary to determine the bacteriological profile of admitted patients in a hospital setting, and also determine the bacteriological profile of hospitalized patients in order to reduce the high rates of amputation and in-hospital mortality rate in people with diabetes and foot ulcers (14). Patients with diabetes have a 12–25% lifetime risk of developing a foot ulcer. Diabetic foot ulcers have increased the public health care awareness and its associated morbidities, patient's quality life impairment, and the implied costs for management have attracted the attention of numerous health care providers (15).

Case report

An 86-year-old male presented to the emergency department with a chief complaint of an ankle injury and a foot non-healing ulcer sustained from a drill accident 2 months prior to his visit in our hospital. The patient also related that he had diabetes for approximately 13 years without any treatment to control the disease. During the initial consultation, the patient related no use of any antibiotic therapy for his non-healing wound to the right foot. The patient was admitted to the hospital and further clinical and medical imaging revealed the presence of a neuro-ischemic ulcer complicated with infection and osteomyelitis (Fig. 1). The presence of vascular compromise and osteomyelitis were diagnosed by the vascular surgeon on call through the macroscopic visualization of a large area of tissue necrosis, accompanied by abscess and radiological analysis respectively. Initial laboratory analysis included a complete blood count, accompanied by a fasting glucose level which was requested shortly thereafter. The patient's hemoglobin was 8.8 mg/dL (reference value: 11.0–16.5 mg/dL), hematocrit was 25.8% (reference value: 42–54%) and white blood cell count was $23.5 \times 10^3/mm^3$ (reference value: $3.5 - 10.0 \times 10^3/mm^3$) with granulocyte percentage equivalent to 85.6% (reference value: 43.0–76.0%). The differential count showed a segmented neutrophil percentage of 80% (reference value: 54–62%/2,700–6,200/mm^3), and 1% of eosinophils (reference value: 2–5%/100–500/mm^3). The random plasma glucose level corresponded to 305 mg/dL (reference value: 70–99 mg/dL). Automated equipment (Abbott Cell-dyn1700 Diagnosis and Bioplus BIO2000) were used to obtain the results of the complete blood count and glucose levels, respectively.

At the time of the patient's admission, a broad coverage of intravenous (IV) meropenem (500 mg/12 h) and IV vancomycin (1 g/12 h) were initially administered empirically for 9 days in order to prevent any clinical and systemic signs of sepsis. On the ninth day of hospitalization, there was a progression of the infection with the patient having systemic symptoms including increased body temperature and diaphoresis. A consultation was then made for wound debridement and obtaining of wound specimens for bacterial cultures and antibiogram. Before the clinical specimen was collected, the wound was thoroughly cleaned and irrigated by using 0.85% sterile sodium chloride solution, and subsequent drying it with sterile gauze, thus preparing the site for physical debridement. A sterile and disposable scalpel was then used for this procedure, in order to remove all necrotic tissue that enclosed the injury. After this stage, the wound was again irrigated with a 0.85% sterile sodium chloride solution

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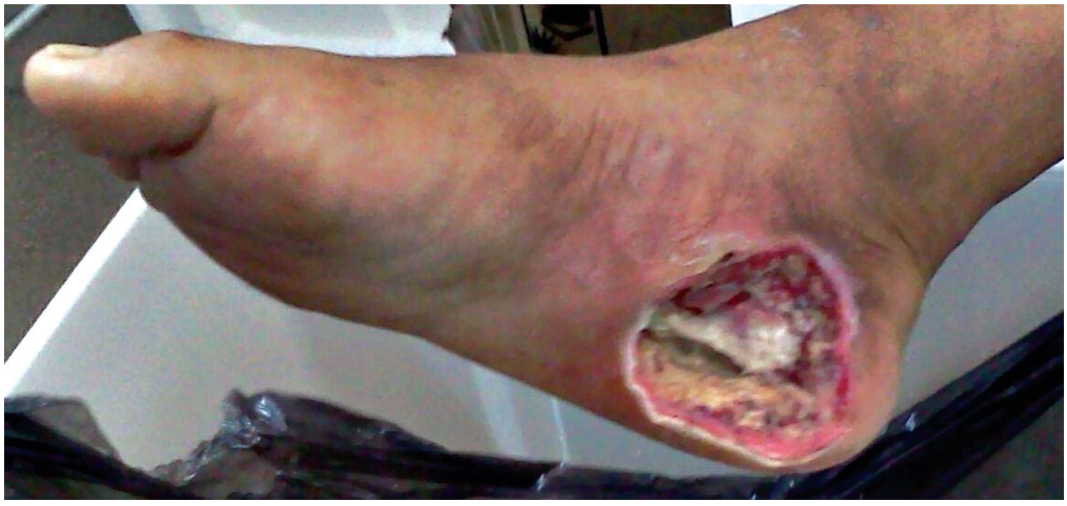

Fig. 1. Eighty-six years old diabetic patient with an infected neuro-ischemic ulceration and osteomyelitis.

allowing for the collection of the specimen with the aid of a sterile culture swab that was immediately placed in Stuart transport medium (Difco Laboratories, Detroit, MI, USA) and sent to the Laboratory Mycobacteria (LABMIC), Brazil, for processing.

Upon arrival at LABMIC, the clinical specimen was duly processed using protocols recommended by Murray et al. (16). The specimen was then seeded in selective MacConkey agar, blood agar, and liquid culture medium brain heart infusion (BHI) broth (Difco Laboratories, Detroit, MI, USA), followed by inoculation in conventional atmosphere at 35°C for 24 h. Biochemical tests were then performed to identify bacteria at the species level. Minimum inhibitory concentrations of vancomycin and tigecycline were determined by *E*-test (AB biodisk, Solna, Sweden), while susceptibility to cefoxitin, cefepime, ceftriaxone, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, oxacillin, gentamicin, imipenem, rifampicin, tazobactam-piperacillin, tetracycline, and trimethoprim/sulfamethoxazole was determined using the disk diffusion test. All assays were performed in accordance with clinical and laboratory standard institute guidelines (17–19). *Staphylococcus aureus* (ATCC 25923) was included as control strains. In order to establish a confirmation of the oxacillin-resistant strain, a Mueller–Hinton agar supplemented with 4% sodium chloride and oxacillin 6 μg/mL was used, followed by the use of cefoxitin (30 μg) agar disk diffusion.

Final microbiological analysis revealed a polymicrobial infection caused by multi-drug resistant organisms. The identified organisms were *Staphylococcus aureus* resistant to oxacillin, *Acinetobacter baumannii*, and *Acinetobacter lwoffii*, all classified as multi-drug resistant if resistance was found in 9 out of the 14 drugs tested. Accuracy was verified by the reference laboratory using disk diffusion on Mueller–Hinton agar (17–19). Due to these results, the patient was transferred to another hospital on the 25th hospitalization day, with the recommendation of lower extremity amputation.

Discussion

According to Carvalho et al. (20), some patients with type 2 diabetes can remain for 10 years or even longer with the disease before the diagnosis can be made by the usual onset's symptoms. This fact explains many cases where the first manifestation is the appearance of a chronic complication. In this case study, the duration of diabetes reported by the patient was approximately 13 years. Another aspect that should be taken into consideration was the patient's denial and lack of knowledge regarding his disease and the importance of proper foot care. Knowing that the existence of an ulcer could be the start of an infection in the diabetic foot, the need to classify it according to its severity was made necessary. The Wagner's classification which was chosen in this case study was classified as 5, characterized by the presence of the foot's extensive gangrene, with necrotic tissue and soft tissue infection. The diabetic foot international consensus (21) recommends the clinical specimen collection by aspiration or biopsy of deep tissues. In this case study, we used the procedures recommended by Johnson et al. (22) and Sapico et al. (23). According to Wheat et al. (24), who evaluated these procedures, the culture swab or curettage collection provides the best results with regard to the isolation of the greater number of bacteria when compared to the collection made by suction, and when the lesions are superficial or deep, with cellulitis but no abscess (22).

The bacterial species isolated in this study were oxacillin-resistant *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Acinetobacter lwoffii*, all with multi-drug resistance standard phenotype. Oxacillin-resistant *Staphylococcus aureus* strains represent a chronic problem in Latin American hospitals. Data from the SENTRY

program revealed that 30–50% of *Staphylococcus aureus* strains isolated in hospitals are resistant to oxacillin (23). Carvalho et al., upon analyzing the resistance profiles of isolated *Staphylococcus aureus* strains, showed a profile of 11.5% (20). In addition, the patients had all undergone hospitalization once or more in the last 6 months, and some were taking antibiotics at the time of collection. Until the late 1970s, there was little evidence of the spread of oxacillin-resistant *Staphylococcus aureus* strains in the general population. However, since the 1980s, several reports of oxacillin-resistant *Staphylococcus aureus* infection in patients who had not been hospitalized nor had contact with staff working in hospitals were published (20).

It has been shown that the carrier status of oxacillin-resistant *Staphylococcus aureus* persists in some patients for several years, thus, ensuring the spread of these strains, since these patients return to the general population. In addition, there appears to be evidence of oxacillin-resistant *Staphylococcus aureus* spread among local populations and outside the risk groups. Further studies are required to determine the state of dissemination of these strains in the community (24). An important fact about our case report is that the collection of the specimen for culture was only performed on the ninth day of hospitalization and although the patient had reported not having been admitted in the previous 6 months, the colonization could have occurred in the same hospital.

This case report also identified two species of Acinetobacter's genus (*Acinetobacter baumannii* and *Acinetobacter lwoffii*), both showing the phenotypic pattern of resistance to all classes tested according to the recommendations of the clinical and laboratory standards institute (non-fermenting) for this bacterial genus. The *Acinetobacter lwoffii* is generally commensal and is present in normal skin microbiota in approximately 25% of healthy individuals (25). However, *Acinetobacter baumannii* is the genus' species most often associated with nosocomial infections and is rarely found in human skin microbiota (26). The pathogenic potential of these bacterial species is due to its various virulence factors that allow their survival in the hospital environment as well as the ability to cause disease, particularly in debilitated patients (27).

Bacterial resistance is an issue that is becoming increasingly common in diabetic foot infections. A number of risk factors related to antibiotic resistance have been shown in some studies. This includes previous antibiotic therapy and its duration, frequency of hospitalization for the same wound, length of hospitalization stay, and the presence of osteomyelitis (28). In our case report, the patient reported of not having used antibiotics prior to his injury as well as not having been hospitalized in the previous 6 months. However, at the time of his hospitalization, he received empirical treatment with broad-spectrum antibiotics. Therefore, it could be suggested that the natural selection imposed by antibiotics led to the elimination of susceptible bacterial species (vancomycin and meropenem), leaving only the resistant strains in the damaged tissue (29).

The biofilm's role formation on common chronic wounds or wounds that are difficult to heal with treatment has gained increasing attention in the clinical setting, due to its implication in resistance to treatment and presence of infection. Biofilms represent communities of microbial cells attached both to a surface and to each other and embedded in a matrix and extracellular polymeric substances. These polymeric substances are produced by the microorganisms in order to increase their chance of survival in a given environment (30, 31). There is strong evidence that different bacterial species in these ecosystems join together, amplifying the possible exchange of genetic information. The proliferation of these organisms occurs more slowly, therefore hindering the action of the antimicrobial agent that acts mainly when the bacterial cells are in the process of division. Furthermore, these bacterial cells are much more protected from antibiotics, which have limited access to niches where they are, and from the host response, which in the diabetic patient is already weakened due to peripheral vascular disease and the already installed setting of immunosuppression that is characterized with diabetes (32, 33).

The existence of biofilms greatly complicates the clinical use of antimicrobials, favoring the emergence of resistant bacteria. Multi-drug resistant bacteria are associated with treatment failure and increased morbidity and mortality rates. In the absence of a microbiology laboratory at the center studied, the choice of the antimicrobial agent in the treatment of a diabetic patient with an infected foot ulcer was carried out empirically (34). The need for an etiological diagnosis and treatment follow-up of these patients was noted. Study on antimicrobial resistance in developing countries reported the discovery of high percentages of isolates resistant to antimicrobials and warned against the absence or lack of microbiology laboratories in those countries (23). The implementation of these labs would lead to treatments based on isolating the agent and on determining the sensitivity profile in regards to antimicrobial agents. Empirical treatments could jeopardize the implementation of treatment strategies, seeing as the resistance varies from region to region, leading to insufficient treatment of patients in these countries, as well as to increased costs and morbidity and mortality rates.

Conclusion

Unfortunately, multi-drug resistant organisms have become increasingly common, making empirical therapy decisions more difficult (35). In many cases, empirical

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therapy is necessary, especially in therapeutic centers that have no microbiology laboratories and limited resources. This case report emphasized the need of health care centers and providers for improvements on microbiological laboratories in a hospital or medical center. In addition, periodic antibiotic resistance surveys could also help all health care providers as well as the local population on the best treatment strategies.

Conflict of interest and funding

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