First case report of infection by *Mycobacterium wolinskyi* after mammoplasty in Brazil

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Abstract

Mycobacterium wolinskyi is a rapidly growing mycobacterium, first described in 1999 as a member of the group *Mycobacterium smegmatis (Mycobacterium smegmatis, Mycobacterium wolinskyi* and *Mycobacterium goodii*). Only 19 case reports all over the world have been described on literature, none of them in Brazil. On this report, it is described one case of infection after a mammoplasty procedure performed in a private health service in the county of Recife, Pernambuco, Brazil, in 2009. The mycobacteria specie was identified using biochemical tests and sequencing the specific gene *rpoB*. To treat the infection by *Mycobacterium wolinskyi* it was necessary to combine antibiotics for a long period of time associated with surgical procedures of the breast abscesses.

Case Report

On September 15th, 2009, a 29-year-old woman, 61 kg, 1.69 m stature, Caucasian, with no comorbidities, post-graduated, Brazilian, from Recife-PE was submitted to an elective bilateral reductive mammoplasty on a private hospital of Recife-PE to remove 200 mL of each breast using the *L technique* for resections of excess of skin and breast tissue. Then an ampoule of adrenaline was infiltrated into her breasts, the bandage was realized using saline and Polivinilpirrolidone-iodine. The surgical procedure was concluded in 3 hours. The patient made her bandages at home using water, soap and an antiseptic solution of chlorhexidine gluconate. Healing occurred normally, with no trauma and no presence of inflammatory signs. One year after surgical procedure, on October 17th, 2010, the patient referred edema, heat and pain on her left breast. Although left breast presented no blush and normal aspect of scar.

It was requested a breast ultrasonography (USG) and it was prescribed a non-hormonal anti-inflammatory, Nimesulide 100 mg, one pill a day for 5 days, with no improvement of the signs and symptoms. USG revealed an image of a fluid collection filled by thin echoes, extending from 9 o'clock to 3 o'clock, with an antero-posterior diameter with approximately 2.3 cm, far around 2 cm of the skin with an increase of the echogenicity of the subcutaneous tissue on the region (Figure 1). It was then prescribed treatment with cephalexin, 500 mg every six hours and Nimesulide, 100 mg, one pill a day for 5 days.

As there was no improvement of the clinical conditions, an aspiration of the fluid collection was performed in November 23rd, 2010 on patient's left breast with an entry on the intern superior upper quadrant, obtaining a greenish secretion which was sent for automatized culture and antibiogram, both negative for bacterial growth. After the procedure, it was prescribed ciprofloxacin, 500 mg, 2 pills every twelve hours for 2 days and one pill every twelve hours totaling 10 days, without improvement of the condition. On November 30th, 2010, the patient was submitted to a surgery to drain the breast's abscess, maintaining ciprofloxacin 500 mg, one pill every twelve hours, Diclofenac sodium, 100 mg one pill a day and Dipyrone one pill every six hours for 7 days.

The sample collected in this procedure was sent for automatized culture with antibiogram and for smear tests on acid fast bacilli (AFB), both showing negative results.

After the end of the treatment with antibiotics, on December 13th, 2010 an USG showed an increase of the echogenicity on the cellular subcutaneous tissue and on the breast's fat, associated with 2 collections which presented debris in suspension and irregular and inaccurate contours, measuring: 8.8×3.1×1.7 cm (vol =24.2 cm^3), located on the superior upper quadrants of the left breast and another with 2.2×1.2×0.8 cm (vol=1.1 cm^3); deeper than the previous one, which was located on the transition of the left lower quadrants, presenting 2 reactive lymph nodes on the left axilla, measuring 1.8 cm and the 1.4 cm, respectively.

After confirming the presence of the collections, another aspiration was performed using USG, on the same breast in December 14th, 2010, and it was also requested in a private laboratory of the city. Another culture and cytological exam of the collected sample, showed one inflammatory cyst and growth of AFB on a specific culture medium, but the mycobacterium specie was not identified due to fungus contamination on the sample.

A Chest X-ray was requested, and it didn't show abnormalities, another USG was performed on January 24th, 2011, which revealed a new fluid collection, homogeneous, measuring: 2.0×0.7×1.5 cm located on the internal superior upper quadrant of the left breast. It was then prescribed vibramycin, 100 mg, one pill every twelve hours and trimethropim sulfamethoxazole, 400/160, one pill every twelve hours for 6 months, and it was also requested a new drainage. The drained material was sent to the Public Health's Central Laboratory – Dr. Milton Bezerra Sobral (LACEN-PE), being isolated non-tuberculous mycobacteria in the culture medium. *Mycobacterium wolinskyi* was identified by sequencing specific genes; this technique was performed at Aggeu Magalhães Research Center, FIOCRUZ-PE. As the patient presented an evident improvement of the clinical conditions, the treatment scheme proceeded for more 6 months, independent on the antibiogram's result (Table 1) remaining

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asymptomatic for almost 11 months.

After this period, on January 9th, 2012, the inflammatory signs and symptoms reappeared on the left breast, an USG showed 4 cystic images, the biggest at 12 o'clock measuring: 0.7×0.6 cm; the second had slightly thick walls associated with hyperechogenicity of the cellular subjacent subcutaneous tissue at 1 o'clock, measuring: 0.7×0.6×0.5 cm, far 1 cm of the skin and about 4 cm of the nipple; the third cyst presented an heterogeneous content with two adjacent cysts, located at 10 o'clock and measuring: 1.4×1.3×1 cm and 1.3×1.1×0.8 cm, far 3 cm of the nipple and 2 cm of the skin; and the fourth image was located at 5 o'clock measuring: 2.9×1.9×1 cm, far 1 cm of the skin and 4 cm of the nipple. The patient was then submitted to a new surgical procedure to drain the collection and to withdraw the necrotic tissue. This tissue culture revealed one more time the presence of *Mycobacterium wolinsky*, identified by sequencing specific genes. The prescribed therapy was an association of antibiotics, initially under hospital regimen, amikacin 1 g injectable per day with ciprofloxacin 500 mg every twelve hours and doxycycline 100 mg. Amikacin was maintained for 10 weeks under domiciliary regimen, 1 g intramuscular 3 times a week. The other 2 classes of antibiotics were also maintained for 6 months. After this period the patient was released from the therapeutic scheme with complete regression of the clinical symptoms.

Methods

Bacteriology

The bacilloscopy performed with the samples was negative for AFB. The culture on Löwenstein-Jensen medium revealed AFB growth on less than 7 days, suggesting RGM. The colonies did not show any coloring, they were resistant to the para-nitrobenzoic acid (PNB) and to the Hydrazide of the 2-carboxilic acid (TCH); they did not show rope spoilage and the test for the presence of niacin was negative.[1]

Partial sequencing of the rpoB gene

A 764-bp fragment was amplified and sequenced with primers MycoF (5_-GCAAGGTCACCCCGAAGGG-3_) and MycoR (5_-AGCGGCTGCTGGGTGATCATC-3_).[2] A total of 5 µL of each DNA solution (50 µg/mL) was added to 45 µL of a PCR mixture containing 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 2.5 mM MgCl2, 200 µM each dNTP, 1 µM primers, and 1.0 U of *Taq* DNA polymerase (Promega). PCR mixtures were heated at 95°C for 1 min and then subjected to 35 cycles of denaturation at 94°C for 30 s, annealing at 64°C for 30 s, and extension at 72°C for 90 s, with a final step of 72°C for 5 min. Amplicons were purified with GFX PCR DNA and a Gel Band purification kit (G&E) and sequenced in an ABI PRISM 3100 sequencer with a BigDye Terminator cycle sequencing kit (Applied Biosystems). The sequences found were edited and aligned by analyzing the sequencing electropherograms using the program BioEdit v7.0.9. The sequences obtained were compared with those deposited in the GenBank database by using BLAST (http://www.ncbi.nlm.nih.gov/BLAST). The isolate had partial sequence of the rpoB gene with 99% (683/689) similar to GenBank accession number AY262743, which corresponds to Mycobacterium wolinskyi type strain ATCC 700010. The *in vitro* susceptibility test to antibiotics was performed using the microdilution broth assay (MIC) (Table 1).[3]

Table 1. Antimicrobial susceptibility for *Mycobacterium wolinskyi* isolated on January 24th, 2011.

Drugs	MIC (µg/mL)	Interpretation
Amikacin	4	S
Cefoxitin	64	I
Ciprofloxacin	1	S
Clarithromycin	16	R
Doxycyclin	4	I
Moxifloxacin	≤2	I
Tobramycin	32	R
Sulfamethoxazole	≥64	R

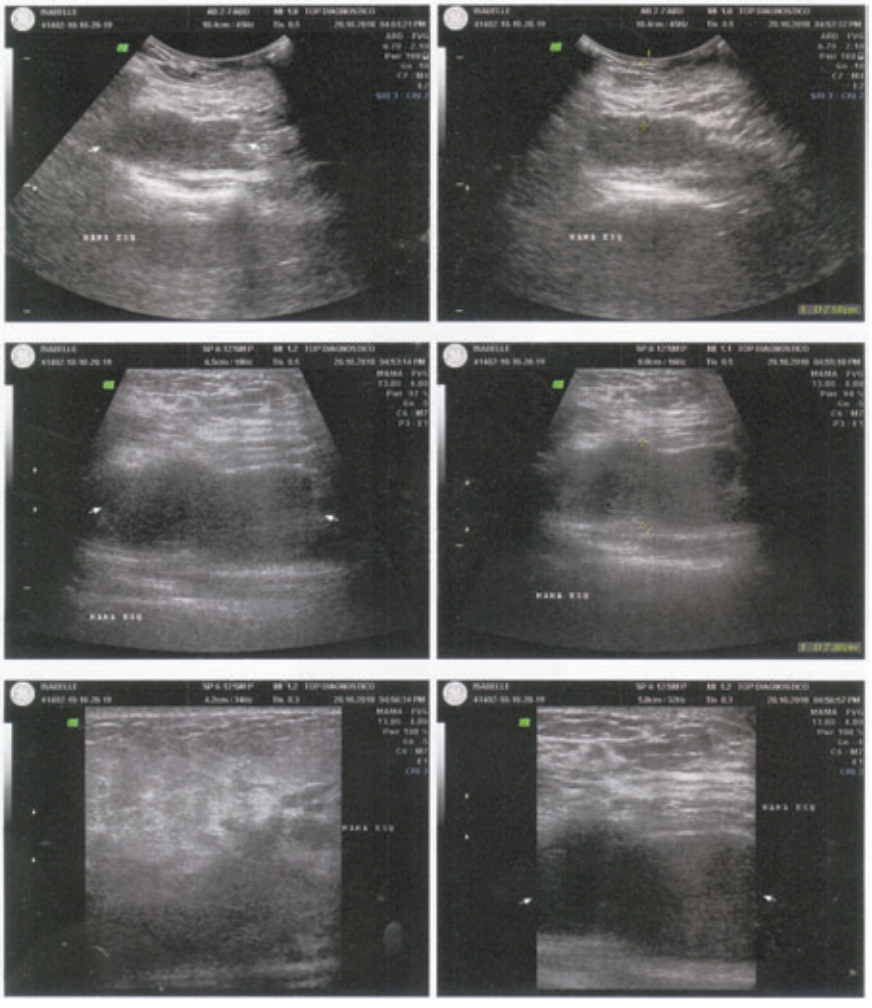

Figure 1. Left breast's ultrasonography showing fluid collection.

Discussion and Conclusions

Among the rapidly growing mycobacteria (RGM), *M. wolinskyi* belongs to *M. smegmatis* group; it was identified for the first time by Brown *et al*. (1999)[4] sequencing 16S rRNA region. The smegmatis group is composed by *M. smegmatis*, *M. wolinskyi* and *M. goodii.* RGM are broadly distributed on the environment, particularly on soil and water, including potable water, biofilms on water distribution piping, swimming pools, sewage and surfaces.[5]

Since its taxonomic description, 19 cases of human infections all over the world were described until now. The majority of infections are post-traumatic and post-surgical, there are no reports of infection after breast aesthetics surgery.[6-8]

Over the last few decades, the majority of the notified cases after infection post mammoplasty are associated with *Mycobacterium fortuitum* (57%) and with *Mycobacterium abscessus* (15.2%). *Mycobacterium wolinskyi* was isolated in only 2 notified cases, representing 2% of all breast infections.[9]

The infection caused by these microorganisms can appear weeks or months after the surgery,[6] with no standard scheme to treat the infection by RGM due to *in vitro* variability and susceptibility of bacteria species. Therefore, it is necessary to identify properly every sample and to determine its sensitivity to antimicrobial agents.[10,11] The choice of the most suited treatment depends on the mycobacteria species involved in the infection, on the clinical presentation and on the patient's immunological condition. These bacteria are capable of producing biofilms, which makes their resistance to antibiotics easier.[12] In general, procedures of drainage, debridement contribute to the resolution of the cases when associated with antibiotic therapy.[6,8]

The typical profile of the *in vitro* susceptibility of *Mycobacterium wolinskyi* is: susceptibility to amikacin, imipenem and trimethropim sulfamethoxazole; resistance to tobramycin; intermediate susceptibility to doxycyclin and ciprofloxacin; and susceptibility to cefoxitin and clarithromycin.[13] Its resistance to tobramycin is a feature that distinguishes *Mycobacterium wolinskyi* from the other members of *Mycobacterium smegmatis* group.[4]

The present case is the first report of infection by *Mycobacterium wolinskyi* after mammoplasty in Brazil. There was no notification of outbreaks during this period at the hospital where the surgical procedures were performed. It is more likely that the bacteria infected the breast at the moment of the mammoplasty, since the presence of inflammatory signs (edema and pain) and abscesses were detected one year after the surgery, with no breast trauma or piercing in the region in this period. It was also observed the absence of comorbidities, presenting similar evolution with the cases which are associated with this type of mycobacterium described on literature.[6,8] To achieve cure, it was necessary to perform several drainage procedures of the abscesses combined with long term therapy using antibiotics, anti-inflammatories and analgesics. This case reassures the occurrence of postsurgical infections by non-tuberculous mycobacteria which must be considered, by health professionals, an important cause of morbidity for human beings.

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