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Granulomatous infection of the hand and wrist due to *Azospirillum* spp.

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ABSTRACT

We report a case of *Azospirillum* infection manifestating as granulomatous tenosynovitis of the right hand, in an immunocompetent middle-aged female. We highlight the unusual source of the infection, the diagnostic workup, as well as the treatment approach.

1. Case report presentation

A 50-year-old Caucasian female presented to the outpatients department with signs and symptoms of inflammation of the right hand. She had been stung on her right thumb, while she was cleaning shrimps 6 weeks before.

Two weeks after the incident, a collection developed under the nail and progressed with redness and swelling of the right thumb. A 2-week course of oral ciprofloxacin (500 mg, bd) was unsuccessful in controlling the symptoms, and the patient deteriorated with pain, redness, and swelling of the hand and numbness of the ulnar border of right forearm. The rest of her medical history was clear. She had the habit of frequent gardening as an outdoors activity.

On admission, laboratory investigations showed white blood cell count of $7.5 \times 10^3/mm^3$ (neutrophils 56%, lymphocytes 35%, and eosinophils 4%), erythrocyte sedimentation rate of 15 mm, and C-reactive protein of 28.4 mg/L (reference values, 0–5 mg/L). An ultrasound examination of the right wrist showed fluid collection over the sheath of the flexor tendons and tenosynovitis involving the extensor tendons. There were no palpable axillary lymph nodes. A punch biopsy of the thickened thumb skin showed multiple epithelial granulomas with Langhans multinuclear giant cells, but no necrosis (Fig. 1A) Acid-fast stain was negative. The patient continued to deteriorate on oral ciprofloxacin with progressive pain and hand swelling, extending into the lower part of the forearm. There was widespread crepitus on palpation of the tendons of the flexor apparatus.

One week later, an extensive surgical debridement in association with synovectomy was undertaken, and multiple biopsies were obtained. Histology of tendon sheaths and fragments showed an abundance of granulomatous tissue, with lymphocytic and monocytic infiltrations and regional fibrin depositions (Fig. 1B).

The tissue fragments were homogenized, and direct Gram stain showed clusters of weakly stained Gram-negative rods, which were acid-fast stain negative. Culture of the tissue was performed on McConkey and Sabouraud agar plates and Cooked Meat Broth, incubated at 37 °C under ambient air conditions; Columbia agar supplemented with 5% sheep blood and chocolate agar plates, incubated at 37 °C under 5% CO_2 conditions; Columbia agar supplemented with 5% sheep blood, hemin (5 mg/L), and vitamin K (1 mg/L), incubated at 37 °C under anaerobic conditions; and in duplicate Lowenstein-Jensen medium slants (LJ) for incubation at 37 °C and at room temperature (RT) (all media were obtained from Bioprepare, 16346, Gerakas, Greece). After a period of 10 days, growth was observed only on the LJ slant incubated at RT. The colonies were semitransparent, white, raised, with rough surface. Acid-fast stain was negative, whereas Gram stain revealed Gram-negative pleomorphic rods with enhanced clustering morphology (Fig. 2). Subculturing from the LJ slants on to the above mentioned culture media showed that the microorganism could grow very slowly (5–7 days) on blood agar plates, LJ slants, as well as on Mueller-Hinton (MH) agar plates incubated at 30 °C or RT, but poorly when incubated at 37 °C. The

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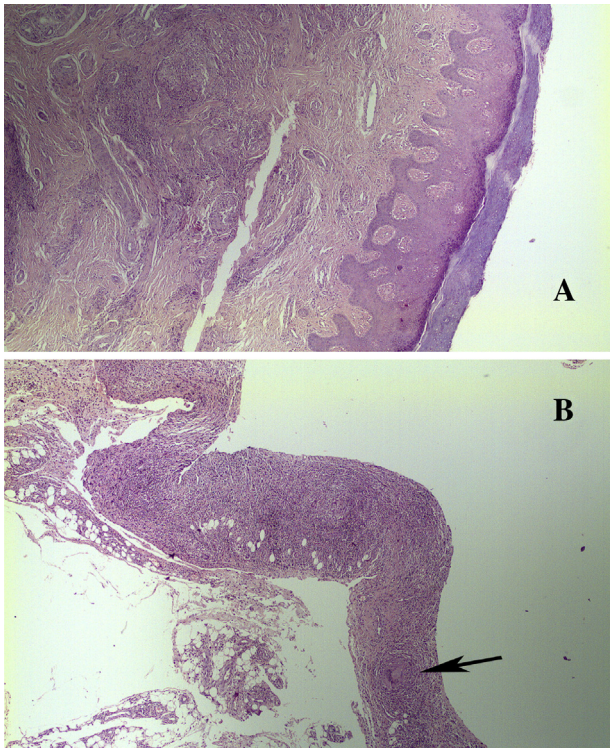

Fig. 1. (A) Thumb punch biopsy with granulomatous inflammation in the dermis; Langhans multinuclear giant cells are present (hematoxylin/eosin stain, magnification ×125). (B) Tendon sheath histology, showing granulomatous inflammation with multinuclear giant cells (arrow), but no caseous necrosis (hematoxylin/eosin stain, magnification ×125).

colonies on blood agar and MH agar plates were pleomorphic, white, semitransparent, smooth, and rough (Fig. 3). They were not pigmented or mucoid.

Preliminary testing for catalase, oxidase, and urease activity and reduction of nitrates were positive. Phenotypic identification using the API 20E or the API 20NE identification systems (bioMerieux SA, Marcy L' Etoile, France) failed to produce an adequate profile. In that respect, 2 previously described 16S rRNA amplification and sequencing protocols were used for genus and species identification (Sipsas et al., 2006; Siala et al., 2008). DNA was extracted from the colonies using the commercially available QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). PCR was performed using the GoTaq Hot Start Colorless Master Mix (Promega Corp., Madison, WI 53711, USA) and a MyCycler Thermal Cycler (Bio-Rad Laboratories MEPE,

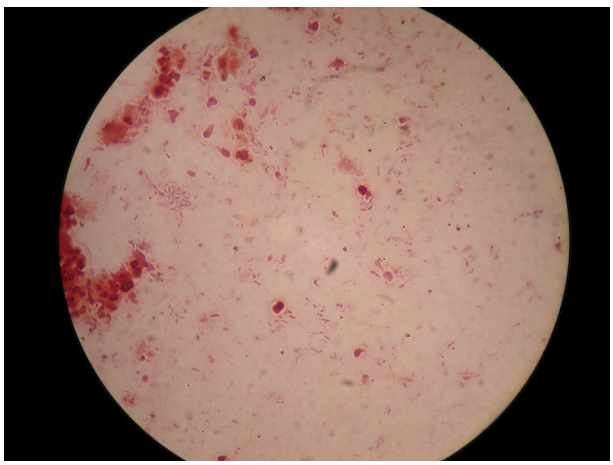

Fig. 2. Gram stain from the culture (Gram stain, magnification ×1000).

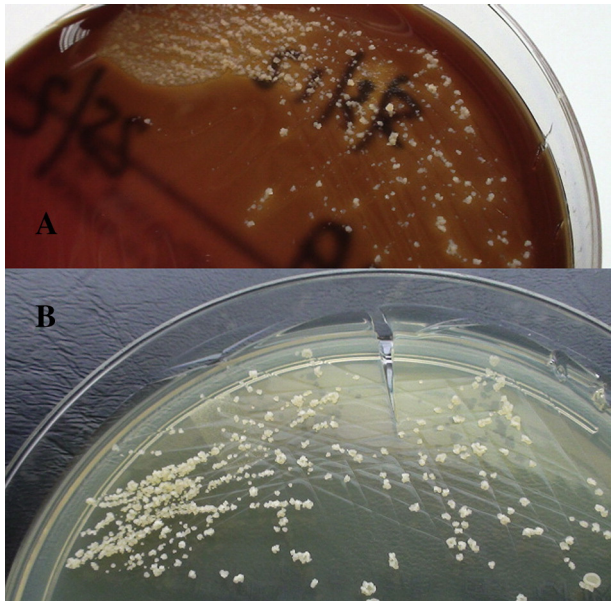

Fig. 3. (A) Growth on 5% sheep blood agar, incubation for 6 days at RT. (B) Growth on MH agar plates, incubation for 6 days at RT.

11527, Athens, Greece), in 50-μL reaction volumes. Purification of the PCR product was performed using the NucleoSpin Gel and PCR Clean-up kit (Macherey Nagel GmbH, 52355, Düren, Germany). Sequencing was performed in a 3130 Genetic Analyzer (Applied Biosystems Life Technologies Ltd, Paisley, PA4 9RF, UK). Sequencing results of the forward and reverse reactions were aligned and edited using the Data Analysis in Molecular Biology and Evolution software ver. 5.1.5 (University of Ottawa, Ottawa, Canada). A consensus 1225-bp sequence result was obtained, and a subsequent BLAST search of GenBank retrieved similarities of 98–99% with various *Azospirillum* spp. strains. Identification to the species level was not possible using the specific protocols. The nucleotide sequence of the isolate has been deposited under the GenBank accession number EU599218.

Antimicrobial susceptibility was determined using the gradient strip method (Etest; bioMerieux, Marcy L' Etoile, France) on 2 media,

Table 1
Comparative susceptibility results as determined using gradient strip method.

Antimicrobial agent	Range[a]	MIC (mg/L)	
		MH[b] agar	SBMH[c] agar
Benzylpenicillin	0.002–32	>32	8
Ampicillin	0.016–256	0.5	0.25
Amoxicillin + clavulanic acid	0.016–256	0.032	0.016
Piperacillin + tazobactam	0.016–256	0.25	0.125
Cefotaxime	0.002–32	>32	0.25
Ceftazidime	0.016–256	4	0.5
Ceftriaxone	0.002–32	>32	>32
Cefepime	0.002–32	>32	>32
Imipenem	0.002–32	0.004	0.004
Meropenem	0.002–32	0.004	0.004
Aztreonam	0.016–256	0.125	0.032
Erythromycin	0.016–256	2	1
Clarithromycin	0.016–256	4	2
Clindamycin	0.016–256	1	0.5
Ciprofloxacin	0.002–32	0.004	0.004
Levofloxacin	0.002–32	0.008	0.008
Doxycycline	0.016–256	0.125	0.5
Gentamycin	0.016–256	0.064	0.016
Tobramycin	0.016–256	0.064	0.016

[a] The concentration range in mg/L of the gradient strip used.
[b] MH agar plates.
[c] MH agar plates supplemented with 5% sheep blood.

MH agar plates and MH agar plates supplemented with 5% sheep blood (SBMH) (Bioprepare, Greece). Incubation of the plates was performed at RT and ambient air for 5 days. Recorded MICs are shown in Table 1 comparatively. No interpretation was performed regarding the susceptible/resistant category, as those microorganisms are not covered in susceptibility guideline documents.

Postoperatively and while waiting for the microbiological results, the patient received a 45-day course of clarithromycin (500 mg bd) on the assumption of *Mycobacterium marinum* infection, even though acid-fast stain was twice negative. Functional recovery was excellent, and so treatment did not change after the final identification and susceptibility testing results became available.

2. Discussion

Granulomatous tenosynovitis may be infectious in origin, caused mainly by nontuberculous *Mycobacterium* spp. (Bartralot et al., 2005). It was first described in patients using swimming pools and often was called "fish-tank granuloma" (Swift and Cohen, 1962). Other infectious causes include histoplasmosis, sporotrichosis, Q-fever, and nocardiosis, while noninfectious associations include sarcoidosis and chronic tophaceous gout (Schwartz, 1989).

The patient described in this case report presented with signs and symptoms of granulomatous tenosynovitis that was attributed to the isolated *Azospirillum* strain. Reports are scarce regarding infections caused by *Azospirillum* spp. (Cohen et al., 2004), and in that respect, a direct comparison with other cases is difficult, although the few cases presented are all infections of the skin and soft tissue as a result of wounds (Cohen et al., 2004), very similar to the one presented here.

These microorganisms have several similarities with the *Roseomonas* genus, which has been described to cause infections among the immunocompromised (Sipsas et al., 2006; Rihs et al., 1993). In addition, there are reports of *Azospirillum* strains from clinical cases misidentified as *Roseomonas* (Cohen et al., 2004), thus raising questions of underdiagnosis and underestimation of this potential pathogenic microorganism. It should be noted, however, that *Azospirillum* is very commonly used in agriculture as a biological fertilizer, especially in wheat, maize, and sugarcane, as it fixes nitrogen (Döbereiner and Baldani, 1979; Mehnaz et al., 2007; Okon and Itzigsohn, 1995; Russo et al., 2008), and it is considered to poses an exceptional safety record. Environmental and agricultural microbiologists are familiar with its features, but its existence is almost unknown to the clinical laboratory, as the media and protocols used in every day clinical practice do not support its optimal growth (as was obvious in the present case report). In that respect, it is possible that, although it is considered a safe microorganism, an infection can be easily missed or misinterpreted, thus explaining the scarcity of similar references in the literature.

With the information we currently have, we cannot exclude the possibility that the *Azospirillum* strain was transferred from the shrimp appendages directly upon injury. However, due to the regular occupation of the patient in gardening, it is also possible that the shrimp injury assisted the entrance, during the gardening process, of the soil bacterium in the deep tissues, thus causing the infection.

The patient's treatment was unsuccessful using ciprofloxacin, albeit the very low MIC that was obtained, but successful with the combination of surgical debridement and clarithromycin, which, nevertheless, had higher MICs than ciprofloxacin. Susceptibility testing of these species presents further difficulties, as it is not covered in current guidelines and no available breakpoints can provide a correlation between MICs and clinical efficacy of the antimicrobial chemotherapy. It should be noted, however, that MIC values obtained on MH agar plates were for some antibiotics one or more dilutions higher than those obtained on SBMH agar, thus indicating a possible better growth in the MH medium. Nevertheless, it is obvious that surgery played the key role in the successful outcome.

To our knowledge, this is the first time a granulomatous infection due to *Azospirillum* spp. is described in an immunocompetent patient. The patient was successfully treated with combination of surgical debridement and antibiotics to an excellent recovery.

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