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Case report

Uncommon finding in a pulmonary graft versus host disease: A first report of Tsukamurella pneumonia in a pulmonary graft-versus-host disease

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ABSTRACT

A 64-year-old woman presented to Our Department with 2 weeks history of fever and cough. Through a series of radiological and invasive diagnostic studies we finally reach an unexpected diagnosis of *Tsukamurella pneumonia*; Diagnosing an ILD is a dynamic process, and that is the reason why complex cases discussed in a multidisciplinary team may need to be reconsidered in light of evolution of the disease and the results of the performed exams with a flexible approach. *Tsukamurella spp.* is an obligate aerobic, Gram-positive, weakly acid-fast, non-motile bacillus that belongs to the order *Actinomycetales.* Pneumonia caused by *Tsukamurella* is exceedingly rare, and only few cases are reported in the literature. Our aim is to evidence the paramount importance of Multidisciplinary team discussion in deciding the most appropriate diagnostic is of and therapeutical strategy.

Introduction

Tsukamurella species are obligate aerobic, gram-positive, weak acid-fast, nonmotile bacilli and belong to the order Actinomycetales. They are found in various environments, such as soil, water and petroleum reservoir wastewater. They are considered rare opportunistic pathogen, because most reported cases have been related to immunosuppression. Reported infections have included pneumonia, brain abscesses, catheter-related bloodstream infections, ocular infections and sepsis. Currently, there is no commercially available test for identification. However, sequence-based identification, including matrix-assisted laser desorption ionization time-of-flight mass spectrometry, is an alternative method for identification. Gold standards for diagnosis and treatment still remain undetermined but newer molecular biological techniques can provide accurate identification and contribute to the appropriate selection of definitive therapy for infections caused by this organism.

Reported management consist in combinations of several antimicrobial agents and the length of treatment has yet to be determined and should be individualized according to clinical response. Favorable prognoses can be achieved through earlier identification of the cause of infection, as well as successful management, including appropriate antibiotic therapy together with source control [1].

Further analyses of cases are necessary to establish the most adequate diagnostic tools and treatment regimens.

Case presentation

A 64-year-old immunocompromised woman presented with a two-weeks history of pyrexia and productive cough. Her family doctor commenced azithromycin and then for a presumed respiratory tract infection (still taking at the time of the first outpatient visit). Fever resolved but cough and general malaise persisted. In view of the incomplete clinical response, she was therefore referred to the respiratory team for further investigations. Her past medical history was significant for multiple myeloma (IgG k subtype), which had been managed with 3 previous allogenic stem cells transplantations (2 allogenic and 1 from healthy volunteer-donor matched for HLA type for recurrency). Last transplantation was successfully performed seven years ago. She had already been diagnosed with chronic graft versus host disease (GvHD) with pulmonary, hepatic, cutaneous, ocular and mucosal

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involvement. At the time of presentation GvHD was in remission, and the patient was not taking any immunosuppressive therapy. Her history was negative HIV, HBV, HCV and Tuberculosis. She had no animal and no environmental exposure. Her white blood cells number was normal together with lymphocyte subsets. She was receiving acyclovir for antiviral prophylaxis. For 3 years she had frequent episodes of acute bronchitis without any bacterial, fungal or viral isolation. Routine blood test showed stable level hypogammaglobulinemia since many years.

Physical examination and findings

The patient appeared in no acute distress. Vital signs revealed temperature of 36,5 °C, peripheral pulse of 87 beats/minute and blood pressure of 137/86 mm Hg. Oxygen saturation was 97 % on room air. There was no sign of edema neither lymphadenopathy. Respiratory examination revealed scattered bilateral coarse crackles that mobilized with coughing, compatible with airways secretions. Cardiac and abdominal examinations were unremarkable. Laboratory examination on peripheral blood highlighted a mild neutropenia (1.98 ×10 3/ul), mild lymphocytosis (4.32 ×10 3/ul), and a mild monocytosis (1.38 ×10 3 /ul). A chest x-ray demonstrated evidence of a right upper zone nodularity in the sub clavicular area (Fig. 1), and a high-resolution CT scan was therefore arranged.

The latter highlighted a bilateral diffused bilateral nodular pattern with one cavitation (9 mm) in the right superior lobe (Fig. 2); absence of pleural effusion and no signs of lymphadenopathy.

Diagnosis

The patient went on to have a fibrobronchoscopy with bronchoalveolar lavage (BAL). Bronchoalveolar lavage cultures were positive both in Chocolate PolyViteX agar (Fig. 3) and in GVPC (Glycine-vancomycin-polymyxin-cycloheximide agar), a selective medium used for isolation of Legionella spp. from clinical specimens, but that allows the growth of bacteria such as Tsukamurella spp. and Nocardia spp. Colonies grew after 48 h of incubation at 35 °C in 5 % of CO2, presenting an unusual morphology similar to fungal growth. Gram staining showed straight, gram-positive rods. The positivity of bronchoalveolar lavage was confirmed by Ziehl–Neelsen staining, and the presence of Acid-Fast Bacillus was detected by BACTEC MGIT™ 960 (Becton–Dickinson and Company). The isolated strain was identified as Tsukamurella tyrosinosolvens by MALDI-TOF MS with a high level of confidence. The identification was performed by using a modified protocol described by Bagnarino et al. [2] Spectra were acquired in a linear positive ion mode at a laser frequency of 60 Hz across a mass/charge ratio (m/z) of 2000 to 20,000 Da by using the Microflex LT MALDI-TOF MS (Bruker Daltonik GmbH, Bremen, Germany). The protein profiles were obtained through the software FlexControl™ 3.4 and analysed with the program FlexAnalysis™ 3.4. Genotypic identification was performed using 16 S rRNA and GroEL gene sequencing. DNA extraction was conducted with the use of DNeasy Blood & Tissue Handbook – Pretreatment for Gram-Positive Bacteria (QIAGEN). 16 S rRNA gene was amplified by primers LPW3606 (5′-TACTTCGGGATAAGCCTG-3′) and LPW3607 (5′-ACGACTTCGTCCCAATCG-3′) as described by Woo et al. [3] PCR amplification of GroEL gene was performed as suggested by Teng et al. by using the following primers LPW34162 (5′-GACG CTCATCGTCAACAAGATCC-3′) and LPW33894 (5′-GGACTYAGAA GTCCATGCCGCCCAT-3′) [4]. The sequences obtained for 16 S (1309 bp) and GroEL (806 bp) were matched to that of Tsukamurella tyrosinosolvens present in the GenBank database by using the BLAST algorithm with 99 % similarity (http://www.ncbi.nlm.nih.gov/blast). Antimicrobial susceptibility testing was carried out using Sensititre™ RAPMYCO2 Susceptibility Testing Plate (Thermo Scientific™). The

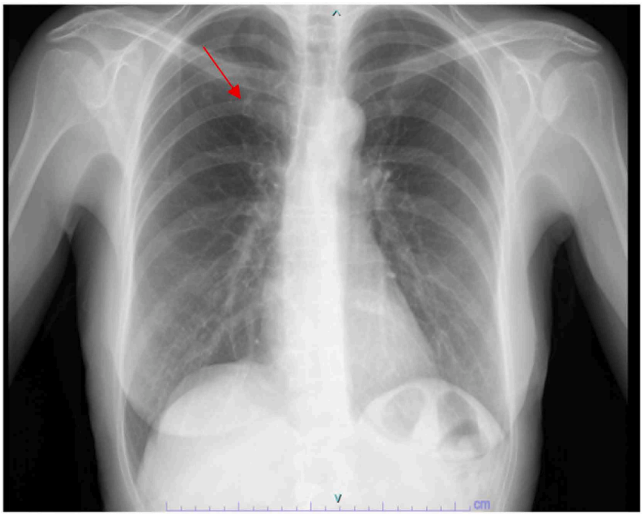

Fig. 1. Right upper lobe cavitation in chest X-ray antero-posterior view.

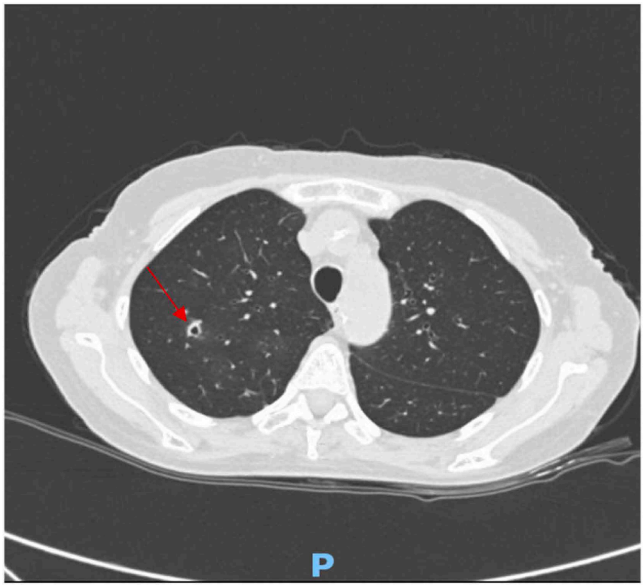

Fig. 2. Right upper lobe cavitation with bilateral nodular pattern in chest CT scan axial view.

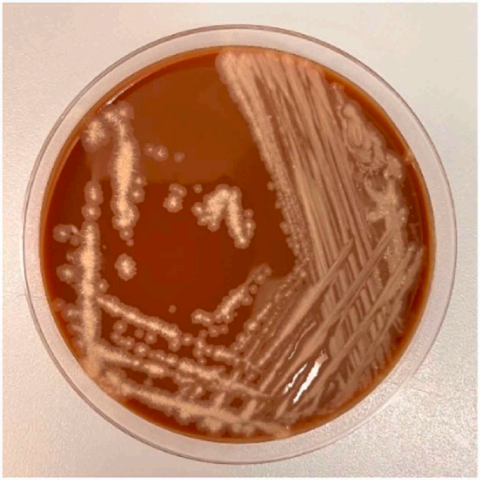

Fig. 3. Bronchoalveolar lavage cultures were positive in Chocolate PolyViteX agar.

strain was resistant to Amoxicillin/ acid clavulanic but was susceptible to Amikacin, Ceftriaxone, Ciprofloxacin, Imipenem, Linezolid, Minocycline, Moxifloxacin, Tobramycin and Trimethoprim/sulfamethoxazole, while Clarithromycin and Doxycycline were intermediate. As no European guidelines are available regarding Tsukamurella genus, common guidelines and breakpoints were used following Clinical and Laboratory Standards Institute recommendations for Nocardia and other aerobic Actinomycetes (Woods et al., 2018) [5].

Treatment and results

The clinical case was discussed at multidisciplinary team level (MDT) with the local microbiologist and infectious disease specialist in view of the mild clinical severity and of the residual symptoms of productive cough, lethargy and weight loss: The patient was discharged home on triple antibiotic regimen treatment of minocycline, ciprofloxacin and nebulized amikacin for 2 months, with outpatient control visit. The patient improved clinically and radiographically over a period of 3 months (Fig. 4). Her fever and cough completely resolved. Repeat computed tomography scan of chest showed remarkable improvement in the area of infiltrate seen on prior imaging with stability of the nodular infiltrates. A repeat fibrobronchoscopy with bronchoalveolar lavage fluid culture performed after 2 months of antibiotic therapy confirmed the absence of *Tsukamurella* isolation.

The optimal management of *Tsukamurella* infections has yet to be determined; in our case the treatment strategy was chosen considering the following aspects:

- Based on treatment principles for nocardiosis and atypical mycobacterial infections, several combinations of antimicrobial agents have been proposed as potential treatments for *Tsukamurella* infections. Consequently, we chose a multidrug regimen considering antimicrobial susceptibility testing.
- The length of treatment for *Tsukamurella* bacterial infections has yet to be determined and should be individualized. Considering that frequent relapses and severe infections are reported, especially in hosts who are immunocompromised we chose to administer prolonged antibiotic treatment based on clinical and radiological response and bronchoalveolar lavage negative results.

Discussion

Tsukamurella spp. is an obligate aerobic, Gram-positive, weakly acid-fast, non-motile bacillus that belongs to the order *Actinomycetales*. Some *Tsukamurella* species are commensal bacteria found in the environment, both in the ground and in water, and are causes of opportunistic bacterial infections in patients with chronic pulmonary disorders or patients who are immunocompromised [6–9].

Pneumonia caused by *Tsukamurella* is exceedingly rare, and so far, only few cases are reported in the literature: most cases are relatively acute to subacute in onset with clinical features of fever, productive cough, haemoptysis, dyspnea and fatigue. Radiographically they have typical consolidative changes that can be bilateral. Cavitary lesions could be present in immunocompromised patients [9–15].

Concerning our specific case treatment: optimal strategy is yet not standardized, mainly due to the rarity of infections and lack of standard susceptibility methods for *Tsukamurella spp*. *Tsukamurella* isolates have tendency to be susceptible to aminoglycosides, macrolides, fusidic acid, imipenem, ciprofloxacin, trimethoprim and sulfamethoxazole, vancomycin, and teicoplanin, but resistant to penicillin, oxacillin, tetracycline, chloramphenicol, and expanded-spectrum cephalosporin. In our case report we decided to treat the patient with a 3 antibiotics regimen based on susceptibility testing (minocycline, ciprofloxacin and nebulized amikacin for 2 months). Management by experts' opinion on a case-by-case basis and a multidisciplinary team approach including the local microbiologist, respiratory medicine team, infectious diseases expertize is of paramount importance.

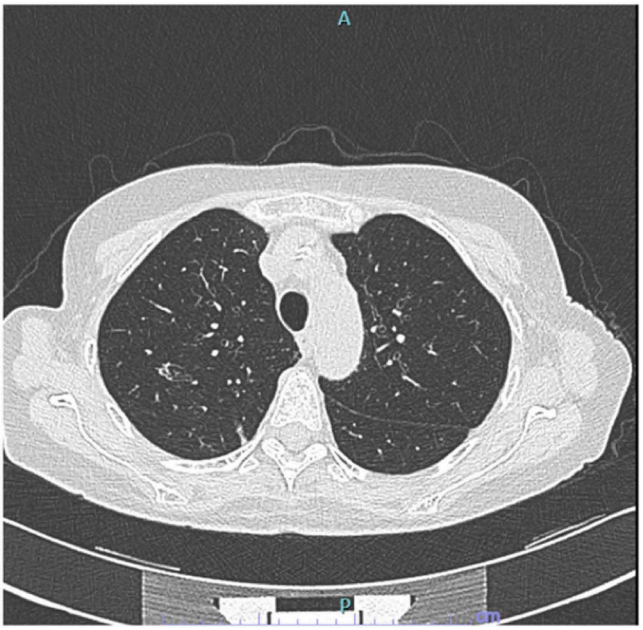

Fig. 4. Resolution of the cavitary area in the right upper lobe in chest CT scan axial view.

The first step towards a reliable diagnosis of *Tsukamurella* infections is a high level of suspicion (especially in immunocompromised host) based on subtle symptoms and radiological features compatible with opportunistic infection. Tsukamurella spp. are environmental saprophytes that can behave as opportunistic pathogens in presence of predisposing factors. Furthermore, Tsukamurella spp. may be responsible for respiratory infection due to the delay in microbiological identification fostering the initial consideration of these Gram-positive bacilli as contaminant microorganisms; hence the need to provide the correct identification of the microorganism in the shortest possible time. In the reported case, the use of MALDI-TOF technology allowed to provide the correct identification at the genus and species level. In case of nonavailability of reference spectra, molecular methods with different molecular target genes such as 16 S rRNA and groEL is suggested, even if 16 S rRNA is not discriminative enough to provide a species-level identification. Alternatively, in designated laboratories, WGS can be a useful tool, with its superior accuracy and database-driven operations.

Conclusions

- To our knowledge this is the first case of *Tsukamurella* infection reported in chronic pulmonary GvHD disease.
- The organism is difficult to differentiate from other related species, and it is also difficult to determine its antimicrobial susceptibilities, consequently gene sequencing is recommended when available.
- Multidisciplinary team discussion is of paramount importance in deciding the most appropriate diagnostic and therapeutical strategy especially in such-cases when clinical and radiological data are aspecific.

Declaration of Competing Interest

All authors declare that they have no conflicts of interest.

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Guarantor: F.R. Bertuccio had full access to all of the data in the

study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Contributions

N. Baio, V. Chino, S. Montini, V. Conio, A. Cascina, L. Pisanu, P. Putignano contributed substantially to the data analysis and interpretation, and the writing of the manuscript.

M.Siciliano, J. Bagnarino, V. Monzillo, D. Barbarini contributed as expert Microbiologists by performing genotypic identification and gene sequencing.

G. Stella and A.G. Corsico: Supervision.

Author Agreement Statement

All authors declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

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