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ACTINOMYCES GRAEVENITZII PULMONARY ACTINOMYCOSIS AT IBN ROCHD UNIVERSITYHOSPITALCENTER, CASABLANCA(ABOUTTWOCASES)

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Abstract

Pulmonary actinomycosis is a rare, often under-diagnosed, chronic indolent infection caused by Actinomycesspecies, a Gram-positive anaerobic bacterium. Only a limited number of cases have been published in theliterature describing pulmonary infections caused by Actinomyces graevenitzii. The aim of our work is to reporttwo cases of pulmonary actinomycosis caused by A. graevenitzii, a species isolated for the first time in ourLaboratory,andtodeterminethecontributionofmassspectrometry(MALDI-TOF/MS)initsidentification.

CASEPRESENTATION

1- Mr S.O, aged 61, presented to the Pneumology Department at the Ibn Rochd University Hospital inCasablancawithchestpain andaprogressivedrycough,evolvinginacontextofapyrexiaandadeclineingeneralconditionfor4 months.Hismedicalhistoryincludedchronicsmokingandalcoholism.

Chest CT images revealed multiple cystic lung lesions, with peribroncho-vascular thickening in the right upperlobe associated with micronodules and scissural thickening. A. graevenitzii was isolated from the culture of abronchial aspirate, performed by flexible bronchoscopy, and identified by mass spectrometry (MALDI-TOF).The patient began antibiotic treatment with Amoxicillin Clavulanic Acid 1 gram three times daily by mouth for 3weeks. His clinical condition improved. He was discharged on oral amoxicillin clavulanic acid with aconsultationappointmentwithin2months.

2- Mrs D.F, aged 55, presented to the Pneumology Department at the Ibn Rochd University Hospital inCasablanca with dyspnoea and chest pain, all of which had been evolving in a context of apyrexia and astheniafor 2 months. Her medical history included hypothyroidism treated with Levothyrox and pulmonary and hepatichydatidosis. She was admitted to hospital with multiple diffuse cystic pulmonary lesions associated with a mixedimageoftherightupperlobeonchestCT.

Abronchialaspirationwasperformedbyflexiblebronchoscopy, andtheculturewaspositiveforA. graevenitzii,which was identified by mass spectrometry (MALDI-TOF/MS). The patient received the same treatment as ourfirstpatientandherclinicalconditionimproved.

In order to make a proper diagnosis of pulmonary actinomycosis, it is important to emphasise the role ofbronchoscopyinobtainingagoodqualitysampleandtheroleofMALDI-TOF/MS inidentifyingA.graevenitzii.

Keywords:PULMONARYACTINOMYCOSIS,GRAEVENITZIIACTINOMYCES,ANAEROBIA,BRONCHOSCOPIA,BRONCHOALVEOLARWASHING,AMOXICILLIN

INTRODUCTION

Pulmonary actinomycosis is a rare, indolent, slowly progressive disease that accounts for 15-20% ofactinomycosis cases. It is a widespread, suppurative, granulomatous bacterial infection caused by an anaerobicGram-positive bacterium *Actinomyces spp*. Actinomycosis is often underdiagnosed and appropriate treatmentis delayed due to the non-specificity of the clinical and radiological signs of the disease on the one hand, andthedifficultyofidentifyingthebacteriumfromclinicalsamplesontheother.(1)

A number of *Actinomyces* species have been described, andphylogenetic studies using 16S ribosomal RNAsequencing have shown that there are over 30 species in the *Actinomyces* genus, six of which are generallyconsidered to be pathogenic to humans: *Actinomyces israeli*,*Actinomyces gerencseriae*, *Actinomycesnaeslundii, Actinomyces odontolyticus, Actinomyces viscosus*and *Actinomyces meyeri*(2). However, only a smallnumberofcaseshavebeenpublisheddescribingpulmonaryinfectionsdueto*Actinomycesgraevenitzii*(3).

The aim of our work is to report two cases of pulmonary actinomycosis caused by *Actinomyces graevenitzii*, aspecies isolated for the first time in our Laboratory, and to determine the role of mass spectrometry (MALDI-TOF/ MS)inidentifyingthis species.

CASEPRESENTATION

- Case N°1 : Mr. S.O, aged 61, presented to the Pneumology Department at Ibn Rochd Universityhospital center (UHC) in Casablanca, with progressive chest pain, dry cough and headache, evolving in a contextof apyrexia and declining general condition for 4 months, with no other associated signs, notably nohaemoptysis, His medical history includes : smoking (15 packs/year) chronic alcoholism and hashishconsumption, The patient does not have any other infections, chronic or allergic diseases. He washospitalized for multiple bilateral balloon release lung lesions associated with a left cerebral lesion with masseffect.

On admission, the patient was apyretic, tachycardic (105 beats per minute) and normopneic (17 breaths perminute), blood pressure (12/9 mmHg) and oxygen saturation (SpO2 99%, measured by pulse oximetry whilebreathing room air) were normal. Examination of the oral cavity revealed poor oral hygiene. Pleuropulmonaryand cardiovascular examinations were unremarkable. The abdomen was tender, and there was noorganomegaly.Noabnormalitieswerenotedonneurologicalexamination.

C-reactive protein (CRP) was elevated (68mg/L), and other laboratory tests were unremarkable, including bloodcount,renal andliverfunctiontests,andhaemostasis. Repeatedcytobacteriologicalexaminationofsputumwasnegative.

Thoracoabdomino-pelvic computed tomography (CT) images revealed multiple cystic pulmonary lesions, withperibroncho-vascularthickeningintherightupperlobeassociatedwithmicronodulesandscissuralthickening.The search of Mycobacterium tuberculosis in sputum by Bacilloscopy and Culture on Lowenstein-Jensenmedium, as well as Polymerase Chain Reaction (PCR) were negative. Cerebral Magnetic Resonance imagingMRI revealed a 35mm frontal nodular lesion, and another extra-axial frontal lesion enhanced after injection ofcontrast agent probably related to cerebral actinomycosis. Flexible bronchoscopy showed extensiveinflammation localized in the dorsal and apical segments of the right lower lobe, with pus flowing from

bothorigins.

A bronchial aspiration was performed on our patient, whose culture under anaerobic conditions on blood agarsupplementedwith Colistinnalidixicacid(CNA)was positiveafter96hoursincubationat37° (figure1).
Colonies were small, whitish and rough, and Gram staining revealed slightly curved, coryneform Gram-positivebacilli (Figure 2) that did not produce catalase. Identification of the *A. graevenitzii*species was carried out byMALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionisation). These results indicated that our patient's lunglesionwascausedby*A.graevenitzii*.

After identification of the species, the patient began antibiotic treatment with Amoxicillin Clavulanic Acid 1gram three times a day orally for 3 weeks. The patient's clinical condition has significantly improved and CRPlevels normalized (0.99 mg/L), although lung lesions on chest CT were not significantly resolved. The patient wasdischargedonoralamoxicillinclavulanicacidwithaconsultationappointmentin2months.

Figure 1: Blood agar culture of*A.graevenitzii*isolated frombronchialaspiratefrom1st patient

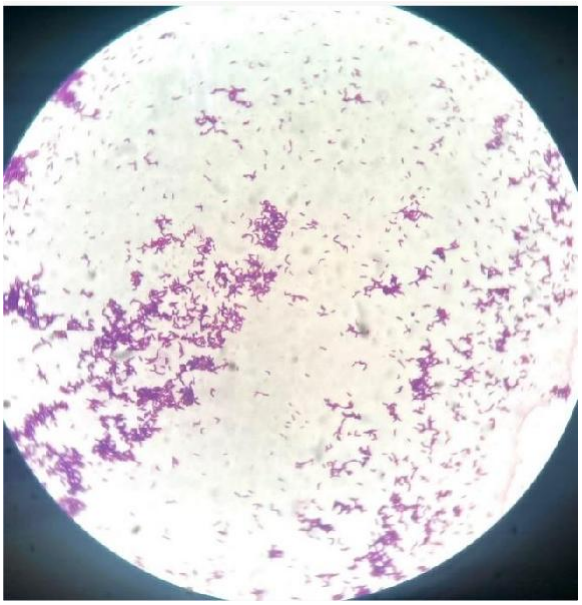

Figure2:Gramstainfrombronchialaspirateculturefof1st patient.

2- Mrs D.F, aged 55, presented to the Pneumology Department at Ibn Rochd UHC in Casablanca withdyspnoea and chest pain, evolving in a context of apyrexia and asthenia for 2 months, with no other associatedsigns. The patient's medical history included Hypothyroidism treated with Levothyrox and pulmonary andhepatic Hydatidosis, with no other particular history. She was admitted to hospital with multiple diffuse cysticpulmonarylesions associatedwithamixedimageoftherightupperlobeonThoracicCT.

On admission, the patient was apyretic, tachycardic (92 beats per minute) and tachypneic (24 breaths perminute), blood pressure (10/6 mmHg) and SpO2 (98%, measured by pulse oximetry while breathing room air)Performansstatus(0)werenormal.Pleuropulmonary and cardiovascularexaminationswereunremarkable.The abdomen was tender with no hepatomegaly or splenomegaly. Examination of the oral cavity revealed poororalhealth.Therestoftheclinicalexaminationwas unremarkable.

A biological assessment was requested: CRP (C-reactive protein), complete blood count, renal and

hepaticfunctiontests,andcoagulationprofilewerenormal.Sputumcytobacteriologicalexamination wasnegative.

The search of Mycobacterium tuberculosis in sputum by Bacilloscopy, culture on Lowenstein Jehnsen mediumand PCR were negative. Tests for Echinococcus granulosus scolex and hooks in bronchial aspirate and hydatidserologywerenegative.

Flexible bronchoscopy showed hypervascularization of the bronchial mucosa, enabling bronchial aspiration tobe performed in our patient, whose culture under anaerobic conditions on CNA blood agar incubated at 37°was positive after 120 hours. Colony appearance and Gram staining of the culture (Figure 3) were identical tothoseofthefirstpatient.Massspectrometry(MALDI-TOF/MS)wasusedtoidentify*A.graevenitzii.*

Once the causative agent had been identified, the patient began antibiotic treatment with Amoxicillin ClavulanicAcid 1 gram three times a day orally for 1 month. The patient has shown marked improvement in clinical status,although the radiological lesions had not been significantly reabsorbed, and she was discharged on oralamoxicillinclavulanicacidwithaconsultationappointmentin2months.

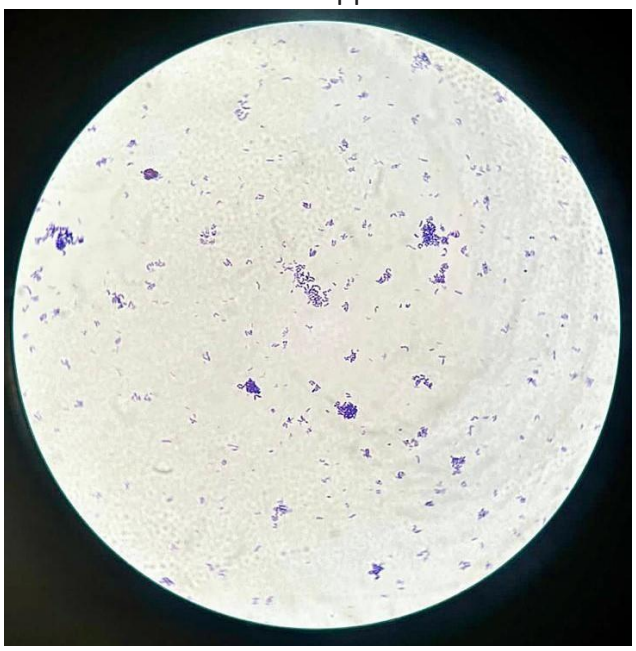

Figure3:Gram stainfrombronchialaspirateculture of 2nd patient.

DISCUSSION

We are currently uncovering an increasing number of instances of pulmonary actinomycosis, despitethe fact that the clinical presentations and imaging characteristics exhibit a lack of specificity. There arefew reports of pulmonary infection by *Actinomyces graevenitzii*first described in 1997 by Ramos et al. onhuman clinical specimens (three respiratory and one bone specimen). (8) In the present study, we describe twocases of pulmonary infection due to this species diagnosed by microbiological identification using time-of-flightlaser desorption/ionization mass spectrometry (MALDI-TOF/MS). Following targeted antibiotic treatment, theclinicalsymptomsandimagingresults

ofourtwo patientsimprovedprogressively(3).

Actinomycosis is a chronic granulomatous disease caused *by Actinomyces* species. *A. israelii*is the species mostfrequently reported in human infections.*A. graevenitzii*is a Gram-positive facultative anaerobic bacteriumbelonging to the human commensal flora of the oropharynx, and colonizing the distal part of the humanesophagus (9), this species isolated almost exclusively from oral or respiratory sites may have a unique abilityto cause clinical actinomycosis. Indeed, *A. graevenitzii*has been identified as a causative agent in pulmonaryactinomycosis (9). Although little is known about its clinical prevalence and pathogenic potential, its frequencyofisolationfromclinicalsamplesisincreasing.(3)

The pathogenesis of *A.graevenitzii*pulmonary actinomycosis remains uncertain, but aspiration oforopharyngeal secretions represents the main source of this infection. In addition, poor oral hygiene, pre-existing dental disease, alcohol abuse, chronic lung diseases such as emphysema, chronic bronchitis,bronchiectasis (3), hematogenous spread or direct dissemination from local infections are other risk factors.(9)Given that the infection can spread to adjacent tissues without respect for normal anatomical barriers, leadingto invasion into the pleura, thoracic wall, or surrounding bone structures, complicated by the formation offistulas and abscesses (9), characterized by small yellow grains or « sulfur granules » measuring 2 to 3 mm indiameter(7).

In our study, the first patient was a chronic smoker and alcoholic with a poor oral condition, the second patientalso had poor oral hygiene in addition to a cystic parasitic infection, Pulmonary Hydatidosis. According to casesin theliterature,onepatienthaddentalcariesandwasan alcoholic(2),anothercasehadperiodontitis(3),andathirdcasesufferedfroma septicoralinfectionwith severalteethmissing(10).Actinomycosiscanoccurinboth immunocompetent and immunodeficient individuals (9). Cohen R D et al. described a case of pulmonaryactinomycosis associated with Infliximab treatment of Crohn's disease (11). A case of disseminated co-infectionwith A. graevenitzii and Mycobacterium tuberculosis has also been reported (12). In addition, S Gliga et al.reportedacaseofpulmonaryinfectionwith *A.graevenitzii*inanimmunocompetentpatient(1).

The clinical manifestations of *A. garevenitzii*pulmonary actinomycosis are often non-specific, represented byfever, cough, dyspnea or chest pain. (3) According to the literature, the typical clinical presentation of *A.garevenitzii*infection is cervico-facial actinomycosis. However, pulmonary localization may occur in 20-40% ofcases(4).
Imaging findings of pulmonary actinomycosis caused by *A. graevenitzii*may present with different aspects suchas a mass, nodules, patchy infiltrates, segmental consolidation of the airspace, or cavitation. On computedtomography, the presence of low central density within a parenchymal consolidation, associated with adjacentpleural thickening, is characteristic (3). This presentation constitutes a differential diagnosis with pulmonarycancer,tuberculosis, coccidioidomycosis oratypicalpneumonia(3).

*A.graevenitzii*is a facultative anaerobic filamentous bacterium of the order Actinomycetales belonging to theclass Actinomycetes, family Actinomycetaceae and genus Actinomyces (Figure 4), characterized by Catalase,Urease, Nitrate reductase, esculin hydrolysis, negative beta-hemolysis, ferments sucrose, andappears asa 0.4to 1.0 µm gram-positive bacillus, straight, curved or pleomorphic, isolated or grouped in pairs, clusters or shortchains. It is an intracellular, non-acid-resistant germ, immobile, does not form endospores and grows well inblood medium on Columbia agar enriched with 5% of horse blood, optionally

supplemented with nalidixic acidor containing sodium carbonate (1). Cultures incubated at 37° and maintained anaerobically are examined after2 days. *A. graevenitzii*has a faster growth time (48 h and 96 h) than other *Actinomyces* species, up to threeweeks (2). This characteristic may explain its different clinical presentation from that of other *Actinomyces*species.(1)

On culture, colonies appear opaque, white, rough and irregular, with a central crater. On agar, examination ofyoung colonies shows branched filaments (4). The failure of bacterial culture in actinomycosis caused by *A.graevenitzii*could be due to several factors in common with other *Actinomyces* species, including: actinomycesgrowth requiring strict anaerobic conditions, the predominance of saprophytic flora which inhibits actinomycesculture, or imperfect sampling such as failure to observe asepsis, transport in an aerobic environment, lack oforientation on the part of the bacteriologist, or previous use of antibacterial treatment. Bacteriologicaldiagnosis is certainly difficult, but its cost-effectiveness can be improved by sampling under good asepticconditions,rapidtransportinananaerobicenvironmentanda specificrequestto thelaboratory(4).

For the identification of *A. graevenitzii*, bacterial cultures and histopathological features of biopsy specimensare essential for diagnosis. However, it is difficult to confirm the presence of bacteria by culture due to priorantibiotic treatment, growth of concomitant organisms or inadequate conditions (13). Consequently, clearcommunication of suspected Actinomycosis with the microbiology laboratory is necessary. With theadvancement of techniques, more and more bacterial identification methods are being used, such as PCR and16SrRNAgene sequenceanalysis,next-generationsequencing(14),orMALDI-TOF/MSmassspectrometry.(2)

Long-term beta-lactam antibiotic therapy is required for patients with pulmonary actinomycosis. Intravenousadministration of penicillin G is recommended for 2-6 weeks, followed by oral administration of penicillin V oramoxicillin for 6-12 months. Surgery may be required to drain large abscesses, marsupialize chronic sinus tractsandexcisefibrouslesions(1).

According to an Italian study conducted by G. Facchin et al, published May 1, 2023, described 2 hematologypatients with *A. graevenitzii*pneumonia, the first patient followed for bone marrow aplasia, the second isfollowed for acute myeloblastic leukemia (AML), In both cases, the infection was diagnosed by bronchoalveolarlavage (BAL) culture with molecular identification by MALDI-TOF/MS. The first patient was treated withAmoxcillin Clavulanic Acid (1g/ 8h) and Isavuconazole 200 mg/d for 3 months with good clinical improvement.The second was treated with Amoxicillin-Clavulanic Acid for 4 months but died as a result of her haemopathy(5). SmarandaGliga (2014) et al, described a case found in a 35-year-old male resident of France, *A. graevenitzii*was isolated from BAL and identified by Polymerase Chain Reaction (16S ribosomal DNA restriction fragmentanalysis),thepatientwastreated withAmoxcillinwithgoodclinicalimprovement(1). YuanYuanetal,inChina,

reported the case of a 47-year-old patient presenting with mucopurulent sputum, dyspnoea and fever. Afterflexible bonchoscopy and bronchoalveolar lavage, *A. graevenitzii was* isolated. MALDI-TOF/MS was used forpathogen identification, the patient was put on Piperacillin Sulbactam for 10 days during hospitalization andthen continued on oral Amoxcillin Clavulanic Acid for 3 months with good clinical improvement (2). AnotherChinese study reported the case of a 75-year-old patient who presented with a dry cough and whose culture ofActinomyces from a BAL came back positive. The pathogen was identified by MALDI-TOF/MS and 16S rRNAsequencing. The patient was started on 6,000 IU ampicillin IV for 1 week, followed by 1.5g amoxicillin orally

for2months,withmarkedclinicalimprovement(3).

Case /Author/Year	**Age /Sex**	**Comorbidities**	**CTSCAN Thoracic**	**Withdrawalpurpose Diagnosis**	**Identificationmethod**	**Treatment**	**Duration**	**Evolution**
Case 1:G. Facchin(2023) (5)	44 years /F	Medullaryaplasia	Lungcondensationin the rightupper lobe(posterior part)	Bronchoalveolarlavage	MALDI-TOF/MS	Amoxicillinclavulanic acid(1g/ 8h) andisavuconazole200mg/d	3 months	Goodimprovement
Case 2:G. Facchin (2023)(5)	64 years /F	Acutemyeloblasticleukemia	Lung nodule intherightposteriorupperlobe	Bronchoalveolarlavage	MALDI-TOF/MS	Amoxicillinclavulanic acid(1g/ 8h)	4 months	Deathfollowing AML
Case3: Smaranda Gliga(2014) (1)	35 yrs/M	No medicalhistory	Excavation ofthe middlelobe	Bronchoalveolarlavage	PCR	Amoxicillin(6g)	6 weeks	Goodimprovement
Case 4:YuanYuan(2022) (2)	47 years /M	Smoking andchronicalcoholism		Bronchoalveolarlavage	MALDI-TOF/MS	Piperacillin-sulbactam (10days)Amoxcillin-clavulanicacid	7 months	Goodimprovement
Case5: DaisukeHimeji(2018) (3)	75 years /M	Chronicsmoking,Guillain-Barrésyndrome,Periodontitis	Excavation oftheright upperlobe	Alveolarbrushing andtrans-bronchialbiopsy	MALDI-TOF/MSAnd 16S rRNAsequencing	Ampicillin IV(6000 mg/1week) thenAmoxicillin(VO) (1.5g/day)	2 months	Goodimprovement
Ourstudy:CASE1 (2023)	61 yrs/M	Smoking andchronicalcoholism,Druguse	Cystic lesions,peribronchovascularthickening oftheright upperlobe	Bronchialsuction	MALDI-TOF/MS	Amoxicillinclavulanic acid3g/d	1 month	Goodimprovement
CASE2 (2023)	55 years / F	Hypothyroidism , Pulmonary andhepatic hydatidosis	Diffuse cysticlesions of therightupperlobe	Bronchialsuction	MALDI-TOF/MS	Amoxicillinclavulanic acid3g/d	1 month	Goodimprovement

Table1:CasesofActinomycesgraevenitziipulmonaryactinomycosisreportedintheliterature.

CONCLUSION

For a proper diagnosis of pulmonary actinomycosis, it is important to note the role of bronchoscopy inobtaining a good-quality sample, and that of MALDI-TOF/MS in identifying *A. graevenitzii*. The overall mortalityofpatientssufferingfrompulmonary actinomycosisismorecloselyrelatedtotheirterrainandhistory.

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