Case Report

Fulminating fungal sinusitis caused by *Valsa sordida*, a plant pathogen, in a patient immunocompromised by acute myeloid leukemia

AYSE KALKANCI*, SEMRA KUSTIMUR*, GULSAN TURKOZ SUCAK**, ESIN SENOL†, TAKASHI SUGITA‡, GERARD ADAMS§, GERARD VERKLEY¶ & RICHARD SUMMERBELL‖

*Department of Microbiology, **Department of Haematology, and †Department of Infectious Diseases, Gazi University, Faculty of Medicine, Ankara, Turkey, ‡Meiji Pharmaceutical University, Department of Microbiology, Tokyo, Japan, §Michigan State University, Department of Plant Pathology, East Lansing, Michigan, USA, and ¶Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands

We describe a case in which a patient immunocompromised by acute myeloid leukemia experienced acute fulminating invasive fungal sinusitis followed by pneumonia and then death. Though the microbiology of the pneumonia could not be directly investigated, nasal lesions revealed fungal mycelium. *Valsa sordida* was consistently cultured from a biopsied sample. The fungus was identified to the genus level based on morphology in culture and DNA sequence homology, and then was placed at species level by means of phylogenetic analysis of the nuclear ribosomal internal transcribed spacer region. The fungus is in the order Diaporthales, family Valsaceae in the Ascomycota and is distributed worldwide as a pathogen of trees in the genera *Populus* and *Salix*. Koch’s postulates were demonstrated to apply in a neutropenic rat model. The fungus was susceptible to antifungals with MIC-0 scores of 0.0313 μg/ml for amphotericin B, 0.25 μg/ml for voriconazole, 0.0313 μg/ml for caspofungin, and MIC-2 of 16 μg/ml for fluconazole. This is the first substantiated report of an isolate in the genus *Valsa* (anamorph *Cytospora*) being identified in human disease.

**Keywords** plant pathogen, nuclear ribosomal internal transcribed spacers, sinusitis, Turkey, *Valsa sordida*

**Introduction**

Sinusitis can be caused by viruses, bacteria or fungi. Fungal sinusitis manifests as a wide range of clinical syndromes. The disease is classified into five major categories: (1) acute invasive fungal sinusitis, (which in very rapidly progressing, imminently life-threatening cases is referred to as ‘fulminant’); (2) chronic or indolent fungal sinusitis; (3) chronic invasive fungal sinusitis; (4) fungus ball (occasionally also termed ‘mycetoma sinusitis’), but note the longstanding international agreements to use ‘mycetoma’ only in reference to subcutaneous infection [1]; and (5) allergic fungal sinusitis [2,3]. Recently, after detailed study, the term ‘allergic fungal sinusitis’ was criticized as misleading, and ‘eosinophilic fungal rhinosinusitis’ was introduced to replace it [4]. This condition was shown via surgical investigation of chronic sinusitis sufferers to be much more common than previously believed in otherwise healthy, atopic individuals [4].

The primary etiologic agents of allergic fungal sinusitis include melanized (dematiaceous) ascomycetes
anamorphs such as Bipolaris, Curvularia, Drechslera, Exserohilum, and Alternaria spp. as well as hyaline anamorphs such as Aspergillus and Fusarium spp. [5]. The principal etiologic agent of fungus ball is Aspergillus fumigatus, while the fungi identified in indolent and chronic invasive sinusitis commonly include the melanized genera listed above as well as Aspergillus spp. The etiologic fungi identified in most cases of acute invasive sinusitis include the zygomycetous fungi Rhizopus arrhizus, R. rhizopodiformis and Absidia spp., as well as certain fungi of ascomycetous affinity such as Aspergillus spp., Pseudallescheria boydii and Fusarium spp. [5]. Most cases of acute sinusitis are viral in etiology; however, when acute fungal sinusitis occurs, it is most often fulminating. Fulminating fungal sinusitis, the most clinically dramatic form of acute invasive fungal sinusitis, is a relatively rare manifestation that usually occurs in immunodeficient patients and that is associated with high mortality and rapid progression, as seen in the case described below [2,3].

Fungal spores and conidia are very common in normal, inhaled air, and are present at numbers hundreds of times the frequency of other biological particles such as pollen grains [2]. Very few of the species present are capable of causing sinusitis. The fungi commonly causing mycoses of the sinuses mainly belong to two ecological categories: some, such as A. fumigatus, P. boydii and the thermotolerant Rhizopus species, are 'compost fungi', typically associated with composts and very warm soils or tepid, stagnant fresh waters, while others, such as the human opportunists in the genera Bipolaris, Curvularia and Alternaria, are pathogens or early successional decay organisms of plant shoots [6]. In the present case report a fungus in the latter category, a plant pathogen in the genus Valsa (anamorph Cytospora), is demonstrated as having been involved in an aggressive sinusitis that was shortly followed by the death of the patient. No member of this genus has previously been implicated in human disease.

Delimitation of species of Valsa is currently under reassessment because morphological features are often unreliable for identification [7,8]. More precise concepts of species are being inferred from phylogenetic analysis of DNA sequence. The accessions of DNA sequences in the National Center for Biotechnology Information (NCBI) repository, GenBank, are not comprehensive for Valsa species. Therefore, this report includes a phylogenetic evaluation that supports the identity of the etiologic agent as Valsa sordida Nitschke.

Case report and methods

Case report

A 55-year-old woman presented to our medical center complaining of weakness and lethargy. The patient was hospitalized on 10 September 2003 in the haematology unit with a diagnosis of Acute Myeloid Leukemia (AML). Before the episode the patient did not report diabetes mellitus, use of immunosuppressant drugs, HIV, or other identifiable risk factors. Anti-leukemic treatment began immediately with 3 days of a 30-min infusion of idarubicin (Zavedos®, Pfizer Inc., New York, NY, USA) at 12 mg/m²/day combined with 7 days of a 24-h daily infusion of arabinosylcytosine (Cytosar-U®, Thomas Micromedex, Greenwood Village, CO, USA) at 1000 mg/m²/day. Antibacterial antibiotic therapy included intravenous piperacillin sodium and tazobactam sodium (Tazocin®, Wyeth-Ayerst, Madison, NJ, USA) combined with amicasin sulphate (generic) at 1000 mg/day. Four weeks after the start of anti-leukemia treatment, fever, headache and sinusitis were noted. Standard and high-resolution computed tomography (CT) scans demonstrated a heterogeneous opacification in the maxillary sinus and a necrotic lesion on the meatus of the maxillary sinus in the nasal cavity. On 7 October material was removed from the lesion by the endoscopic transnasal route. The collected material was biopsied, examined directly by microscopy, and cultured. KOH preparations and haematoxylin and eosin-stained tissue sections of the nasal lesion showed numerous narrow hyaline, septate hyphae. Culture results are detailed below. On 8 October antifungal treatment was initiated using amphotericin B deoxycholate (Fungizone®, Bristol-Myers Squibb Company, New York, NY, USA) at 1 mg/kg/day. On 15 October the patient developed pneumonia. After poor response to therapy was noted, voriconazole (VFEND®, Pfizer Inc.) at 4 mg/kg/12 h was added to the antifungal therapy on 18 October; and the Amphotericin B treatment was discontinued (<1 g total). Antibacterial therapy was changed to intravenous meropenem (Meronem-IV®, Wyeth-Ayerst, Madison, NJ, USA) at 1000 mg/8 h three times a day. The patient died on 22 October. No postmortem examination was allowed. Involvement of fungi in the final pneumonia could not be investigated.

Isolation and cultivation

Tissues of the patient and the test animal (detailed below) were cultured on four replicate Sabouraud dextrose agar (SDA) plates in order to isolate the filamentous fungi seen in direct examination. Four
blood agar and four eosin methylene blue agar plates were used for isolation of bacteria. The plates were incubated at 24°C and 35°C. Microscopic examination was made every 24 h. An unidentifiable, nonsporulating fungal isolate was sent to one of us (T.S.) for nuclear ribosomal internal transcribed spacer (ITS) region sequence analysis. For further investigation the isolate and its ITS sequence data were sent to the Centraalbureau voor Schimmelcultures (CBS). The fungus was subcultured to oatmeal agar (OA [9]) with an embedded sterile Urtica (nettle) stem for 3 wk incubation at 24°C under 12 h cool white fluorescent light/24 h to induce sporulation. The isolate and the existing sequence were sent to one of us (G.A.) at Michigan State University for analysis in the context of ongoing biosystematic study on Valsa.

Standard antifungal sensitivity assays were inoculated with hyphal segments prepared following the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) Approved Standard Method M38-A guidelines [10], incubated at 35°C and examined daily after 48 h for 72–96 h. This method used microplate wells containing broth of Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco Invitrogen, Carlsbad, CA, USA) with L-glutamine, phenol red, and 0.2% glucose buffered with morpholine propane sulfonic acid (MOPS) to pH 7.0. Sensitivity to amphotericin B, voriconazole, caspofungin and fluconazole was evaluated by measuring turbidity in the wells, scored (using the standard scale from 0 to 4) with the aid of a reading mirror and compared to turbidity of the growth control [10]. Endpoints for the minimal inhibitory concentrations (MICs in μg/ml) were determined by selecting the lowest concentration of antifungal compound with a score of 0 (MIC-0, optically clear) except for fluconazole where the endpoint was the lowest concentration with a score of 2 or lower (MIC-2, ca. 50% reduction in turbidity, visually).

Koch’s postulates

A female Wistar albino rat weighing 250 g was used as the sole experimental animal. Neutropenia was induced by using 5-fluorouracil at a dose of 200 mg/kg. The drug was injected subcutaneously seven days before the experimental fungal inoculation. The freshly transferred and actively growing V. sordida was inoculated intranasally on the seventh day of neutropenia. Response to this challenge was examined by recording of symptoms, followed by biopsy of sinus and lung tissues, direct microscopic examination (KOH), culturing on fungal and bacterial media, and direct PCR amplification with panfungal primers (described below). The rat was maintained in the animal facilities at Gazi University under conditions consistent with care guidelines of the Gazi University Ethical Committee.

DNA methodology for sequence-based identification

Total genomic DNA was extracted from mycelium by a cetyltrimethyl-ammonium bromide (CTAB) method [11]. Hyphae were ground to a fine powder in liquid nitrogen with a pestle. One to 2 ml of the extraction buffer was added and mixed. The extract was then centrifuged to remove solids, purified with phenol:chloroform:isoamyl alcohol (24:24:1) extractions and precipitated with isopropanol and centrifugation. DNA was extracted from biopsied tissue with the QIAamp tissue kit (QIAGEN, Hilden, Germany) following manufacturer’s instructions. Samples were obtained from the patient’s paranasal sinuses by biopsy, and from paranasal sinuses and lungs of the test animal at autopsy. Tissue (10 mg) was lysed with proteinase K and incubated in a solution of 25 mM NaOH and 0.5% sodium dodecyl sulfate at 95°C for 10 min, then neutralized with HCL [12], and further processed with the methodology of the kit instructions.

The ITS sequence of the case isolate was compared for homology to the NCBI GenBank database using BLASTN [13]. Following preliminary identification to genus, based on homology to NCBI accessions, the isolate was further compared to a more extensive database of nuclear ribosomal DNA sequences containing 142 sequences from unique isolates of species in the genus Valsa. The database encompassed Valsa isolates collected worldwide from diseased and healthy trees and herbaceous plants. It included sequences from reference cultures of species identified by experts in *Valsa* taxonomy including Défago [14,15], Spielman [16], Adams *et al.* [7,8], and others. Reference cultures were obtained from CBS, as well as from the International Mycological Institute collections (IMI, CABI Biosciences, Egham, Surrey, UK), and the American Type Culture Collection (ATCC, Manassas, VA, USA). Many but not all cultures are referable to accessioned herbarium specimens.

Approximately 2.5 ng of the total genomic DNA was used per 100 μl reaction mixture for PCR amplification [17]. Panfungal primers used in the amplification of the clinical isolate included ITS1 and LR21 [12,17,18] for the ITS, 5.8S, and 5′-end of the 28S rDNA inclusive of the variable domains D1 and D2. Primers used in the amplifications for the *Valsa* database included ITS1, ITS2, ITS3, and ITS4 for the ITS-rDNA [17]. These cycling reactions were performed in a DNA Thermal Cycler (PE Applied Biosystems, Foster City, CA, USA).
or similar machine using standard protocols. For direct amplifications from tissues, and the cultured case isolate, nested amplifications were performed that included ITS1 and ITS4 in the first reaction followed by ITS1 and ITS2 in the second reaction. Nested cycling reactions were performed using the ‘LightCycler’ system (Roche Molecular Systems, Indianapolis, IN, USA) with fluorescent detection of the amplicon. The ITS1 panfungal primer was labeled with the Light Cycler Red 640 fluorophore and the ITS2 primer with fluorescein according to manufacturer’s instructions. The size of amplicons was recorded.

Two hundred μl of each PCR product were purified by using the DNA binding resin and protocol of Wizard PCR Preps DNA purification system (Promega Corp., Madison, WI, USA). Sequencing was performed using a Taq DyeDeoxi Terminator™ cycle system, the ABI Catalyst 800, and the ABI Prism 373A or 377 fluorescence sequencer (PE Applied Biosystems). Sequencing reactions were carried out using the Big Dye fluorescence labeling sequencing kit (PE Applied Biosystems).

**DNA data analysis**

DNA sequences of the clinical isolate and the isolates of the *Valsa* database were deposited and accessioned in the NCBI, GenBank (see Results). The sequences were aligned with Clustal X 1.81 [19,20] and visually proofread. The ITS region sequences were analyzed as uniformly weighed unordered characters, and as interleave blocks of aligned sequence. Sequences were compared in maximum parsimony analysis [21] involving a total of 143 taxa. Ambiguities in alignment of short segments of sequence were tested experimentally for their effect on topology and bootstrap indices. The analysis was computed using PAUP version 4.0b10 [22] using heuristic searches with the tree bisection-reconnection (TBR) branch-swapping algorithm. The tree with the greatest natural logarithm (LN) likelihood was selected from among the equally most parsimonious trees (MPT), using the Kishino-Hasegawa test [23]. To develop a consensus tree, 1000 heuristic searches [24] were performed by bootstrapping [25]. Confidence intervals for branches on the consensus tree were inserted into the selected MPT. The tree was displayed using TreeView [26].

**Results**

Direct microscopic examination of biopsied nasal tissue revealed extensive filamentous growth of narrow hyphae. Hyphae were hyaline, regularly septate, uniform in width, and regularly branched with acute angle branching. No yeast cells, swollen cells, specialized cells, or sporulation were observed in the tissues. Unfortunately, the material was not photographed or preserved. Cultured tissue on the four SDA plates showed heavy outgrowth of fungal mycelium after 72 h of incubation at 24°C, but minimal growth at 35°C. Approximately 10–15 colonies of fungi per plate were recovered from the sinus tissues. Colonies appeared pale to faintly brownish; aerial mycelium was whitish and scantily produced. Microscopic examination every 24 h consistently indicated that the fungus was nonsporulating on SDA.

The etiologic fungus as seen on OA grew rapidly with entire margins and was colorless to faintly sienna with sparse whitish aerial mycelium. Within three weeks of incubation at 24°C under fluorescent light, conidiomata (asexual fruiting bodies also known as pycnidia) were produced copiously at the agar surface (Fig. 1a). They were 1–2 mm diameter at maturity, subglobose to hemispherical, dark reddish brown approaching black, with a smooth surface (glabrous), and occasionally with whitish hyphae around the apical pore (ostiole). Sectioning of fruiting bodies revealed labyrinthine chambers in the locule. Labyrinthine chambers are a diagnostic feature of the mitotic fruiting structure of the *Cytospora* stage of *Valsa*. In Fig. 1b, conidiomata of an unidentified but related *Cytospora* species are shown in cross-section to provide a schematic illustration of this type of divided chamber. Conidia (Fig. 1c) of the case isolate were released from the ostiole in salmon to orange slimy masses. They were colorless (hyaline), one-celled, curved (allantoid), 3.5–5.0 × 1–1.2 μm, with ends rounded and with a minute droplet near each end, and were produced from branched, multiseptate, conidiophores 15–35 μm long. Conidigenous cells (Fig. 1d) were terminal phialides, tapering towards the apical opening with periclinal thickening and indistinct collarettes, 5–12 × 1.5–2.5 μm.

The NCBI, GenBank accession numbers for DNA sequences of the clinical isolate and the isolates of the *Valsa* database are AB188679 (case isolate), AF192314-21, AF260263-6, and AY347316-80. The sequence of the clinical isolate gave a 94% match in nucleotide-nucleotide BLAST search with *Valsa* sp. It was noted that a fungus related to *Valsa* should form a *Cytospora* anamorph on suitable media.

Phylogenetic analysis of the data set of the clinical isolate and 142 other taxa is represented in Fig. 2. Names formatted in italics are of host genus and of identified fungal species. Names in regular fonts are of the country or region of origin and are followed by a
number. The number represents a unique DNA sequence type that corresponds to one or more isolates. The length of DNA sequence for each isolate, inclusive of introduced gaps to permit alignment of the entire set, was 617 nucleotides with 221 parsimony-informative characters. Maximum parsimony gave a tree of 1218 steps, \( LN = -8038.02 \), and consistency index of 0.416, retention index of 0.830, and a rescaled consistency index of 0.346. The species *Cytospora sacchari* represents the root of the gene tree lineage that includes *Cytospora* anamorphs of *Valsa, Leucostoma* Höhnel, *Valsella* Fuckel, and *Valseutypella* Höhnel. A *Cytospora*-like fungus, with morphology typical of *Cytospora* but with sequences more closely homologous to those of *Diaporthe*, served as the outgroup. It was more closely related to *Valsa* than other known fungi [8]. Ambiguities in alignment had no significant effect on topology and did not alter bootstrap indices by more or less than 0.05. No segments of sequence were excluded from the analyses.

DNA sequence obtained from the case isolate clustered within a clade that contained a representative population of *V. sordida* (anamorph *Cytospora chrysosperma* (Pers.) Fries). The isolate was accessioned into the CBS culture collection as *Valsa* cf. *sordida* CBS 115107.

DNA extracts of tissue samples from the sinus were tested in multiple PCR amplifications and the amplicon sizes of the second nested reactions were recorded. The Light Cycler yielded amplicons of 245 bp for all the nested ITS products. This amplicon size was not referable to *Aspergillus* or any other known agent of mycotic sinusitis. The 245 bp amplicons from tissue were identical in size to the nested amplicon obtained from DNA from cultures of fungi isolated from the tissue (case isolate).

The case isolate of *V. sordida* inoculated into the neutropenic animal caused disease symptoms compatible with those seen in the original case, inclusive of the terminal-stage pneumonia. The animal exhibited fever,

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**Fig. 1** Clinical isolate of *Valsa sordida*. (a) Dark structures are pycnidia (asexual fruiting bodies) as seen on oatmeal agar after 3 wk at 25°C. (b) Longitudinal cross-sections through asexual fruiting bodies of an unidentified *Cytospora* species, schematically illustrates the multiply chambered morphology that is diagnostic for this asexual genus occurring as the anamorph of *Valsa* spp. The case isolate formed similar structures (c) Conidia of the case isolate showing mostly allantoid (curved with rounded ends) shape. Scale bar = 10 µm. (d) Internal conidioma (pycnidial) wall and phialidic conidiogenous structures of the case isolate. Scale bar = 20 µm.
In this diagram of a tree of phylogenetic relationships, the clinical isolate is inferred to be *V. sordida* based on maximum parsimony analysis. The analysis is of nuclear ribosomal DNA (rDNA) sequences of the internal transcribed spacers (ITS) and the 5.8S rDNA gene. Confidence intervals are calculated by bootstrap statistics and placed at the branching points, i.e., 93% at *V. sordida* cluster (clade). This tree is one of the most parsimonious trees having the best maximum likelihood value. A similar tree of phylogenetic relationships, but concerning cankers on *Eucalyptus* trees, has been previously published in *Mycolological Studies* 2005; 52.
anorexia and pneumonia and it became stationary in one corner of the cage by the seventh day of the challenge. The animal was then sacrificed and tissues of nasal sinuses and lungs were collected at autopsy. Direct microscopic examination of biopsied tissue of both sinuses and lungs revealed extensive growth of filamentous, narrow, regularly septe, hyaline hyphae of uniform diameter and acute branching pattern. Tissues of both sinuses and lungs were cultured and numerous non-sporulating fungal colonies, approximately 15 colonies/plate of four SDA plates/tissue, grew within 72 h incubation at 24°C and 35°C. The fungal colonies on SDA were characteristic in appearance to the case isolate. Pathogenic bacteria did not grow on the Blood agar or Eosin Methylene Blue agar plates inoculated with the biopsied tissues from sinuses and lungs. Direct PCR amplification of sinus and lung tissues yielded 245 bp amplicons identical to the size of amplicons from isolated fungal colonies from and the case isolate. It was concluded that the fungus isolated in pure culture from biopsied tissue of both sinuses and lungs of the neutropenic animal model was the same as that originally isolated from the patient.

Antifungal susceptibility assays of the V. sordida isolate provided the following endpoints for MICs of the antifungals tested: 0.0313 μg/ml for amphotericin B, 0.25 μg/ml for voriconazole, 0.0313 μg/ml for caspofungin, and 16 μg/ml for fluconazole. Only the fluconazole value is suggestive of resistance. The MICs were determined as the lowest tested concentrations with turbidity ratings of optically clear (100% inhibition), except for fluconazole with ratings of 50% reductions in turbidity, compared to that of positive controls.

**Discussion**

The incidence of fungal sinusitis, particularly in immunocompromised patients, is increasing. The diagnosis of all types of fungal sinusitis initially requires a high level of suspicion by the physician, because clinical examination is usually inconclusive. Surgical intervention becomes important for diagnosis. The most crucial point in the diagnosis is to confirm the fungal elements in the collected material from the sinuses. This is best accomplished by histopathological analysis. Culture is also valuable, but is not as reliable as histopathology. In particular, in eosinophilic fungal rhinosinusitis, surgical material heavily positive for fungal filaments often fails to grow a culture [27], while insignificant contaminants can readily be grown and potentially misinterpreted as pathogenic, particularly if the mucus-loosening techniques of Ponikau et al. [4] are utilized [28]. On the other hand, correlation of positive culture with ongoing infection is relatively reliable in cases of fulminating, acute sinusitis, where most fungal material present is likely to be recently produced and in healthy condition.

To our knowledge, V. sordida has not been isolated previously as an etiologic agent of human infection. Considerable caution was thus used in verifying the significance of this isolation. The biopsy was performed under the strictest conditions of sterile technique and the obtained material was aseptically transferred to the isolation media. The presence of numerous hyphae on direct examination of the biopsy material and the growth of many consistent colonies on agar plates supported the conclusion that the sinusitis case was caused by fungi. Finally, the presence of the fungus in the clinical material was confirmed with direct PCR of the tissue. The size of amplicons of the fungus from direct PCR of tissue and from isolation matched. Sequence of the isolated fungus was submitted to the GenBank repository (GenBank accession AB188679). With an increasing number of immunocompromised patients, new fungal opportunists are increasing. Diagnosis and management of such infections can be difficult and will require a greater understanding of myological details.

In the *in vitro* antifungal sensitivity tests, the case isolate was exposed to four agents from three chemical classes, the polyenes, the echinocandins, and the azoles. The case isolate was highly sensitive to amphotericin B, caspofungin and voriconazole, and had limited sensitivity to fluconazole. It thus showed the approximate pattern of susceptibility found in the majority of *Aspergillus* species as well as most other ascomycetous filamentous fungi, apart from members of the frequently amphotericin-resistant orders *Hypocreales* (*Fusarium*, pink-spored *Paecilomyces*) and *Microascales* (*Pseudallescheria*), and the often fluconazole-sensitive members of the *Onygenales* (*Coccidioides*, dermatomyces) [29,30]. It should be noted, however, that *in vitro* sensitivity is not always accurately reflected by *in vivo* activity against fungal pathogens [29], and may be particularly problematical in patients with severe immunodeficiencies, as seen in the present case. Invasive fungal sinusitis in immunocompromised patients is frequently caused by agents susceptible to amphotericin B but is nonetheless frequently fatal, though a combination of antifungal therapy and aggressive surgical intervention can promote survival [31].

*Valsa sordida* is classified as belonging to phylum *Ascomycota*, subphylum *Pezizomycotina*, class *Sordariomycetes*, subclass *Sordariomycetidae*, order *Dia- porthales*, and family *Valaceae*. It is a common pathogen of *Populus* (aspen, cottonwood, poplar) and
Salix (willow), world-wide. Occasionally, it occurs on Ulmus (elm), Betula (birch), Sorbus (mountain ash), and Sambucus (elderberry). Several of these woody genera occur in the Ankara area where the present case occurred. V. sordida forms two types of spores. Sexual spores (ascospores) are produced in sexual fruiting bodies in cells called asci. During times of precipitation, asci ooze out of the opening in the fruiting bodies in a gelatinous matrix. They may be rain-splashed or may discharge spores into the air. Asexual spores (conidia) are produced in similar but mitotically reproducing fruiting bodies and ooze out to be rain-splashed. They may be disseminated in wind-blown rain as well as in the many small, aqueous microaerosols produced by raindrop splash events that persist in the air for several hours after a relatively heavy rain. Conidia are produced in great numbers on the bark surfaces on infected trees. Ascospores and conidia of Valsa and similar Diaporthales are not common among fungal spores in air except near infected trees during wind blown rains. North Temperate species of Valsa usually do not grow at 37°C, however, some species from Africa, Australia, Indonesia, South America and Hawaii are found to grow well at 37°C [8]. In in vitro testing, the case isolate grew but had a colony diameter of less than 2 mm after 7 d at 37°C on malt extract agar [9]. An exact correspondence between in vitro and in vivo growth rates is not expected in such cases, and merely demonstrating growth at this temperature is usually considered consistent with isolation as a human opportunist. The extent to which other isolates classified in V. sordida share this tolerance of body temperature is not known.

Sexual fruiting bodies of V. sordida are not formed in vitro and inducing their formation by inoculating plant hosts usually fails. Additionally, the asexual fruiting bodies that do form in culture lack crucial morphological features that are present in nature. Thus, identification to species based on traditional morphology is not possible with isolates in culture or with isolates inoculated to hosts. Morphology in culture is usually sufficient to identify the isolate only to the genus level. Even with natural material, only sexual fruiting bodies have sufficient characters for identification to species; and the sexual state is often absent on infected trees in nature. DNA sequence is the best method for species identification in this genus.

It is increasingly being understood that a surprisingly high number of human opportunistic pathogens are associated with internal growth in plant shoot material, especially in relatively thin woody stems, branches and spines. Apart from the dematiaceous genera mentioned in this article’s Introduction, it was recently revealed that most of the common opportunistic phaeohyphomycosis agents in the genus Phaeoacremonium have a niche in grapevines, kiwi fruit vines, and various shrubs and trees [32]. Similarly, the common phaeohyphomycosis agent formerly called Phialophora richardsiae, now Pleurostomophora richardsiae, is regularly isolated from woody materials [33]. Cladophialophora carrionii, one of the most important agents of chromoblastomycosis, was found to occur as an endophyte of cactus spines in its endemic area [34]. It is likely that growth in the potentially strongly sun-heated habitat of fine woody stems and spines may, like the growth in composts of classic opportunistic moulds like A. fumigatus, tend to select for the thermotolerance that helps to pre-adapt fungi for fortuitous human pathogenesis. When this thermotolerance is combined with an effective mechanism of airborne spore dispersal, whether this involves dry spores or rain-splashed material, a potential agent of fungal sinusitis may be the result – even though, as with V. sordida, the substrate colonized in human disease could scarcely be more different from that on which the fungus normally occurs. Fulfillment of Koch’s Postulates in this report supports our conclusion that V. sordida, an endophytic fungus of plant stems capable of growth at 37°C, has caused a fulminating opportunistic infection of the sinuses (and possibly the lungs) of an immunocompromised patient.

References
Sinusitis caused by Valsa


