Clinical significance of filamentous basidiomycetes, illustrated by the novel opportunist Ceriporia lacerata isolated from the human respiratory tract

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Running title: Ceriporia lacerata from the respiratory tract

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Abstract

The filamentous basidiomycete, Ceriporia lacerata, an agent of white-rot of wood, has never been reported in human disease and its clinical significance is not yet known. We describe 4 patients with respiratory diseases where C. lacerata was implicated in a wide spectrum of clinical manifestations ranging from saprobic colonization to fungal pneumonia. The isolates did not show the morphological characteristics that facilitate recognition of filamentous basidiomycetes such as presence of clamp connections, spicules along hyphae or fruiting bodies. The identity of the mold was confirmed by sequencing ITS 1/4 and D1/D2 regions of the rRNA gene. All of the isolates exhibited lowest MIC of posaconazole and isavuconazole (MIC range 0.06-0.125 µg/ml) followed by itraconazole (MIC range, 0.06-0.5 µg/ml), voriconazole (MIC range, 0.125-0.5 µg/ml) and amphotericin B (MIC range 0.25-1 µg/ml). The infections reported here occurred in patients with pre-existing lung damage induced by tuberculosis or chronic obstructive pulmonary disease. Chronic, sometimes fatal infections by the ascomycete, Aspergillus fumigatus and the basidiomycete, Schizophyllum commune is well-established in the presence of an anatomical pulmonary defect or in the background of immunodeficiency. It is postulated that C. lacerata, a novel opportunist basidiomycete, may be involved in similar pathologic processes.

Key words: Ceriporia lacerata, Basidiomycete, respiratory disease, India
INTRODUCTION

Recent developments in molecular identification of fungi have provided an opportunity to evaluate the clinical significance of molds that were previously discarded as unidentifiable contaminants because of a lack of phenotypic characters and absence of sporulation. Among these are several filamentous Basidiomycetes, which produce snow-white, cottony, rapidly growing colonies, in culture consisting entirely of hyphae. Occasionally, special features such as clamp connections, hyphal pegs, chlamydospores, cystidia or conidia may be present, but morphological identification alone fails to identify with certainty as to which out of the nearly 20,000 described species, prevalent in the environment, could be the one dealing with. Although the number of these species reported from clinical and environmental sources is growing rapidly, molecular data are available for only a fraction of these species. Sequencing of the rDNA Internal Transcribed Spacer (ITS) region may offer a match in GenBank, therefore, an increasing number of taxa are being introduced in medical literature (2, 26, 28). Classically recognized basidiomycetes such as *Schizophyllum commune*, *Coprinopsis* spp. (anamorph: *Hormographiella* spp.), and *Phanaerochaete chrysosporium* (anamorph: *Sporotrichum pruinosum*), possess some morphological characteristics specific for their identification (6, 13, 16, 19, 32, 35, 37). Lately, lesser known members from this class of molds such as *Bjerkandera adusta* (14), *Cyclomyces tabacinus* (22), *Irpex lacteus* (5), *Inonotus (Phellinus) tropicalis* (11, 36), *Phellinus undulatus* (38), *Oxyporus corticola* (3), *Volvariella volvacea* (30) and *Perenniporia* sp. (6) have been incriminated as agents of human disease. In the present communication, another filamentous basidiomycete, *Ceriporia lacerata* isolated from 4 human cases is presented with a discussion on its possible clinical significance.
Case 1. A 55-year-old male farmer from the suburbs of Delhi, on anti-tuberculous therapy (ATT) since 1 week, presented to hospital in June 2011 with complaints of loss of appetite since 2 years, left-sided non-anginal, non-pleuritic chest pain since 1 year and productive cough associated with haemoptysis since 6 months. The patient had never smoked and had no history of diabetes mellitus or of IV drug abuse. However, he mentioned to have received treatment for pulmonary tuberculosis twice in the past. The first episode, 6 months back for sputum-smear negative pulmonary tuberculosis, was treated with oral drugs which he discontinued on his own after 2 weeks while in the present episode, oral isoniazid, rifampicin, pyrazinamide and ethambutol, were started 1 week prior to presentation for sputum-smear negative pulmonary tuberculosis. His haemogram, liver and kidney functions were within normal limits. However, the radiograph of his chest showed the presence of cystic shadows and an inhomogenous opacity in the paracardiac area of the left lower zone. A contrast-enhanced computerized tomogram (CECT) of his thorax done a week later showed centrilobular infiltrates in the superior segment of the left lower lobe and cylindrical bronchiectasis in the inferior segment of the lingula. A diagnosis of left lower-lobe consolidation with lingular segment bronchiectasis, as a sequel of the previous pulmonary tuberculosis was made. Pulmonary tuberculosis was considered the primary diagnosis for his present symptoms, given his previous history of tuberculosis and the high endemicity of the disease in this part of the world. However, he did not show any induration to the mantoux test done with 1 tuberculin unit of purified-protein derivative (PPD) of Mycobacterium spp. (Span Diagnostics, Surat, Gujarat, India) and sputum samples tested negative for the presence of acid fast or aerobic pathogens. Direct microscopy of KOH wet mounts of three consecutive sputum specimens and a BAL specimen revealed hyaline, septate hyphae and isolation of...
multiple colonies of white mold in culture on Sabouraud’s glucose agar (SGA) at 28°C and 37°C after 7 days of incubation. The white mold was later identified as *C. lacerata* and the isolate was assigned accession no. VPCI 1873/11 (CBS 133710) for morphological characterization, molecular identification and antifungal susceptibility testing. The diagnosis of fungal pneumonia due to *C. lacerata* was considered. The patient discontinued follow up and could not be further evaluated.

**Case 2.** A 60-year-old, male, farmer from Delhi, presented to our Institute in August 2011 with complaints of productive cough associated with occasional haemoptysis since 4 years, progressive, exertional dyspnoea since 6 months and low-grade fever since 1 month. The patient consumed a non-vegetarian diet and had no history of high-risk sexual behavior, intravenous drug abuse or diabetes mellitus. Nevertheless, the patient was a smoker who had been smoking about 10 beedis (*Nicotiana tabaccum* coiled in a leaf of *Diospyros melanoxylon*) per day for the past 35-40 years. The patient was emaciated and had poor general health. The haemogram, liver and kidney function tests were within normal limits. The X-ray of the patient’s chest showed consolidation in the right lower zone with ipsilateral pleural effusion. A CECT of his thorax showed consolidation of the posterior basal segment of the right lower lobe with ipsilateral pleural effusion. A diagnosis of chronic obstructive pulmonary disease with right lower-lobe consolidation with ipsilateral pleural effusion, pending aetiologic confirmation, was made. The patient was initiated on empirical antibiotic therapy comprising a combination of amoxicillin-clavulanic acid (625 mg tid) and doxycycline (100 mg bid) for 2 weeks. However, he failed to respond to medical management and was further investigated as described hereunder.

The patient underwent a diagnostic flexible, fibreoptic bronchoscopy 3 weeks later. The endobronchial biopsy was suggestive of chronic bronchitis on histopathology. The bronchial
aspirate was negative for the presence of acid-fast bacilli, aerobic pathogens or malignant cells. Direct microscopy of KOH wet mounts of the BAL specimen revealed hyaline septate hyphae. Cultures yielded multiple, white, cottony colonies of a mold on SGA plates incubated for 7 days at 28°C and 37°C which was subsequently identified as *C. lacerata* (VPCI 1921/11; CBS 133711). Three consecutive sputum specimens from the patient also showed the presence of hyaline septate hyphae and growth of the same fungus. He was diagnosed as a case of fungal pneumonia with pleural effusion. Therefore, the patient was administered itraconazole (200 mg bid) but he suffered from a bout of massive haemoptysis barely 2 weeks after the start of medication and could not be resuscitated. Autopsy was not permitted by his relatives.

**Case 3.** A 44-year-old female, school-teacher from Bihar, India, a non-smoker presented to hospital in July 2011. She reported persistent productive cough, on-and-off haemoptysis for 8 years, progressive breathlessness since 4 years, loss of weight and appetite since 2 months and right-sided chest pain and high-grade fever since 15 days. She also gave a history of Raynaud’s phenomenon and had received ATT many times in the past 7 years. On examination, the patient was found to have pallor and her blood investigations showed hypochromic microcytic anaemia with neutrophilia and hypoproteinaemia. A radiograph of her chest showed cavitating consolidation in the right middle and lower lobes and infiltrates in the left upper and lower lobes along with pleural effusion on the right side. A CECT of thorax done 2 days later revealed pleural based cavitating lesions in the same distribution as seen on the X-ray. Her sputum was negative for aerobic pathogens and acid fast bacilli (AFB) for *M. tuberculosis*. This was followed by an ultrasound of the chest which showed loculated pleural fluid on the right side, which upon thoracentesis was found to be purulent. This fluid was of an exudative nature, with low glucose content (7 mg/dL) and cultures of pleural fluid were negative for aerobic, acid-fast or fungal organisms. Adenosine deaminase (ADA) level
of pleural fluid was 154 U/L (against an upper limit of normal of 24 U/L) thus a diagnosis of
tuberculous empyema was made. Her blood culture was negative. However, repeated sputum
cultures grew *Nocardia* species. The patient underwent a CT-guided fine-needle aspiration of
the pulmonary lesions which on histologic examination showed *Nocardia*, liquefactive
necrosis and an ill formed granuloma. Thus a diagnosis of *Nocardia* pneumonia was made
and the patient was started on intravenous therapy of imipenem (500 mg tid) along with co-
trimoxazole (960 mg tid). She was maintained on co-trimoxazole for the next one month but
her symptoms persisted. Although her radiograph showed resolution of the consolidation, the
pleural effusion increased in amount.

The patient was investigated for other coexisting etiologic agent and three consecutive
sputum specimens were sent for mycological investigation. KOH wet mount of sputum
specimens showed presence of hyaline, septate, branched hyphae. Multiple colonies of a
white mold were isolated in cultures of sputum samples on SGA at 28°C and 37°C which
were later identified as *C. lacerata* (VPCI 1603/11; CBS 133712). By this time, a confirmed
growth of *M. tuberculosis* from her sputum sample was received. Also a repeat aspiration of
the pleural fluid was suggestive of tubercular effusion so the patient’s diagnosis was updated
to *Nocardia* pneumonia with pulmonary tuberculosis and tubercular pleural effusion and she
was started on isoniazid, rifampicin, pyrazinamide, ethambutol in undivided daily doses of
300 mg, 450 mg, 1250 mg and 800 mg, respectively, with daily injections of streptomycin
750 mg intramuscular in addition to the co-trimoxazole. Three months follow up of the
patient revealed no growth of *Nocardia* and *M. tuberculosis* in culture, whereupon
streptomycin and pyrazinamide were discontinued. However fungal culture of the sputum
sample still yielded growth of *C. lacerata*. Finally, ATT was stopped in June 2012 after an
ultrasound demonstrated absence of pleural fluid and a repeat sputum examination was
negative for AFB. Currently, the patient is receiving co-trimoxazole only, and continues to
be on an improving trend. With regards to the isolation of C. lacerata, since the patient showed improvement in her symptoms even without antifungal therapy, it was concluded that this was a case of an asymptomatic colonization of the respiratory tract by this basidiomycete mold and no further therapeutic intervention was undertaken.

**Case 4.** A middle-aged male, government employee had been under evaluation since July 2012 for non-resolving cavitating pneumonia at tertiary-care tuberculosis and pulmonary diseases institute in New Delhi. The patient’s sputum had been repeatedly negative for acid-fast and aerobic pathogens. He had undergone many bronchoscopies but no causal organism could be isolated from bronchial secretions. Finally, BAL and three consecutive sputum samples were investigated to exclude the presence of fungal pathogen. Surprisingly, a white-cottony mold was cultured on SGA at 28°C and 37°C after 9 days of incubation from the patient’s BAL and sputum which was later confirmed to be *C. lacerata* (VPCI 2549/11; CBS 133713). He was treated with itraconazole (200 mg tid) and follow up after 4 weeks showed reduction in cough and other symptomatic improvement. Further follow-up of the patient and response to treatment was not known as the patient could not be tracked. This case might be regarded as a fungal pneumonia since the etiological diagnosis was elusive even after repeated bronchoscopies, the only potential pathogen that could be isolated being the above-stated mold.

**MATERIALS AND METHODS:**

**Mycological Investigations:** Three consecutive freshly expectorated sputum and BAL specimens of the above mentioned clinical cases were processed for direct microscopy and culture. The material was cultured on Sabouraud’s glucose agar plates supplemented with gentamicin (25 µg/ml). One set of inoculated plates was incubated at 28°C and second at
37°C for up to 4 weeks. Colonies of white mold growing on SGA were purified and subcultured on Potato Dextrose agar (PDA) plates and incubated at 28°C for 4 weeks.

**Induction of sporulation on decayed wood:** Thick mycelial suspension of all the isolates of *C. lacerata* was prepared in 0.9 % saline and inoculated on autoclaved bark pieces of *Syzygium cumini* (black berry, Jamun tree, Jambu), 3-4 × 0.5-1 cm and stems of tomato (*Solanum lycopersicum*) 3-4 cm long and were placed on PDA medium in 90 mm culture plates and in 150 ml conical flasks. The inoculated petridishes and flasks were incubated at 28°C in light for 4 weeks.

**Molecular identification:** The identification of isolates was done by sequencing of the ITS rDNA and the D1/D2 LSU regions as described previously (6, 7). Briefly, genomic DNA was extracted with 700 µl Tris saturated phenol chloroform isoamyl (25:24:1) two times followed by a chloroform isoamyl (24:1) extraction and ethanol precipitation. The DNA pellet was dried and resuspended in 75 µl of sterile nuclease-free water and treated with 6 µl (10 mg/ml) of RNase (Sigma-Aldrich, Co., St. Louis, USA) for 1 h at 37°C. DNA was amplified using the ITS-1 (5’-TCCGTAGGTGAACCTTGCGG-3’) and ITS-4 (5’-TCCGCTTATTGATATGC-3’) primers, which amplify the ITS region of the ribosomal subunit, and the NL-1 (5’-GCATATCAATAAGCGGAGGAAAAG-3’) and NL-4 primers (5’-GGTCCGTGTATTCAGACGG-3’), which amplify the ~600-bp D1/D2 region of the large ribosomal subunit (20, 39). Amplified DNA was sequenced in both strands on an ABI 3130XL Genetic analyser (Applied Biosystems, Foster City, CA, USA) using the BigDye Terminator Kit (v3.1, RR100, Foster City, CA, USA). Sequences were aligned using the Sequencing Analysis 5.3.1 software (Applied Biosystems, Foster City, CA, USA). GenBank basic local alignment search tool (BLAST) searches (http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi) were performed for species identification,
which was defined as ≥99% homology with the GenBank database reference sequences.

Query coverage of ≥95% was considered significant.

**Antifungal susceptibility testing (AFST):** AFST of the 4 isolates was performed by CLSI microbroth dilution method (8). The antifungals tested were amphotericin B (Sigma, St. Louis, Mo, USA), fluconazole (Pfizer, Groton, CT, USA), itraconazole (Lee Pharma, Hyderabad, India), voriconazole (Pfizer), posaconazole (Schering-Plough, Kenilworth, NJ, USA now Astellas), isavuconazole (Basilea Pharmaceutica, Basel, Switzerland), flucytosine (Sigma), caspofungin (Merck, Whitehouse Station, NJ, USA), micafungin (Astellas, Toyama, Japan) and anidulafungin (Pfizer). For the broth microdilution test, RPMI 1640 medium with glutamine without bicarbonate (Sigma) buffered to pH 7 with 0.165 mol/l 3-N-morpholinepropanesulfonic acid (Sigma) was used. Isolates were grown on PDA for 5 days at 28°C and shifted to 37°C incubation for next 5 days for sporulation. Final inoculum was adjusted to a density of 1.0–5.0 x 10^4 hyphal fragments/spores per ml by adjusting optical density of 0.13-0.18 at 530 nm using spectrophotometer. Drug-free and mold-free controls were included and microtitre plates were incubated at 35°C for 72 to 96 hrs. CLSI recommended quality control strains, *Candida krusei*, ATCC 6258, and *Candida parapsilosis*, ATCC 22019, and reference strains, *Aspergillus fumigatus* ATCC 204305 and *Aspergillus flavus* ATCC 204304 were included. The MIC end points were read visually which, for azoles and amphotericin B, were defined as the lowest concentration at which there was 100% inhibition of growth compared with the drug-free control wells. For echinocandins, minimal effective concentrations (MEC) were defined as the lowest concentration of drug that led to the growth of small, rounded and compact hyphal forms.

**RESULTS**

Multiple, white, cottony colonies of a mold grew after 7-9 days on SGA plates inoculated with sputum and BAL specimens and incubated at 28°C and 37°C. Microscopically, slide
cultures on PDA after 4 weeks of incubation at 28°C revealed hyaline, septate hyphae. No clamp connections or hyphal pegs were seen up to 4 weeks of incubation. All of the isolates showed dense white, cottony growth on wood pieces of *S. cumini* (Fig 1a) and twigs of *S. lycopersicum* (Fig 1b). *C. lacerata* isolate obtained from case 2 (VPCI 1921/11; CBS 133711) showed sporulation on bark piece of *S. cumini* and lactophenol cotton blue mount revealed spicules along hyphae (Fig. 1c) and ellipsoid basidiospores of 3.5-4.5 x 2.4-2.9 µm. (Fig. 1d).

ITS sequences of the four *C. lacerata* isolate (GenBank accession nos. JX984623 - JX984626) showed 99% homology (query coverage ranging from 95-97%) to 8 *C. lacerata* isolates in GenBank (accession nos. HQ331080.1, HQ331032.1, HQ331074.1, HQ331078.1, FJ462746.1, HQ331033.1, JN628149.1 and JN182908.1). Also, LSU sequences of our four isolates (GenBank accession nos. JX984627 - JX984630) showed 99% identity (query coverage 95-97%) with 3 *C. lacerata* isolates (accession nos. AB566280.1, JF416691.1 and HM595618.1) in GenBank. The isolates VPCI 2549/11 (CBS 133710), VPCI 1873/11 (CBS 133711), VPCI 1603/11 (CBS 133712), and VPCI 1921/11 (CBS 133713) were deposited at the CBS Fungal Biodiversity Centre, the Netherlands. The isolates had lowest MIC of posaconazole and isavuconazole (MIC range, 0.06-0.125 µg/ml) followed by itraconazole (MIC range, 0.06-0.5 µg/ml), voriconazole (MIC range, 0.125-0.5 µg/ml) and amphotericin B (MIC range, 0.25-1 µg/ml). All three echinocandins showed high MECs of 8 µg/ml. For fluconazole and flucytosine the MIC ranged from 4-32 µg/ml and 8-16 µg/ml respectively.

**DISCUSSION**

The filamentous basidiomycetes encountered in clinical settings are conventionally classified into Agaricales and Stereales, which in their natural habitat display macroscopically visible fruiting bodies recognizable as mushrooms and shelf fungi, respectively. Most species described from clinical samples are white rotters on dead wood, assimilating polyaromatic
ligninous hydrocarbons in the substrate, which leaves an overabundance of white, cellulosic material behind. This ecology is noted for the shelf fungi *C. tabacinus* (22), *I. lacteus* (5), *I. tropicalis* (11, 36), *P. undulatus* (38), *O. corticola* (3), *P. chrysosporium* (19), *S. commune* and *B. adusta* (14), as well as for *C. lacerata* described in the present communication. Clinical cases by these shelf fungi almost exclusively concern pulmonary colonization, eventually leading to allergic responses but with limited invasion. Fatal systemic infections due to *S. commune* have been reported (7, 27, 29), but the majority of cases by this fungus are saprobic colonization and allergic sinusoidal or bronchopulmonary disease. Similarly, the recently reported basidiomycete mold *Perenniporia* sp. has been shown to be involved in the formation of a secondary pulmonary fungal ball but not in invasive disease (6). In contrast, recurrent invasive infections, in addition to pulmonary colonization, are observed with the members of Agaricales *H. aspergillata*, *H. verticillata* (9, 13, 15, 16, 21, 32, 33, 35, 37) and in *V. volvacea* (30). These mushrooms have a ruderal strategy (12), colonizing compost, dung, self-heated wood-chips, and similar substrates. The possibility is not excluded that the differences in ecological background of the clinically relevant basidiomycete species determine their invasive abilities in the human patient.

The four cases presented above, though from geographical regions spaced 50-1500 km from each other, have a number of features in common. All patients resided in rural/suburban areas, with farming being the predominant occupation; two were farmers themselves. Three out of four patients suffered from underlying lung disorders, with the possible exception of case 4 where no clinical details were available. Cases 1 and 3 previously had pulmonary tuberculosis, and case 2 had the background of COPD. With regard to the commonly reported pulmonary ascomycete *Aspergillus fumigatus*, it is known that fungal colonies may reside in structural lung defects and emphysemas and provoke invasive infection with the onset of immunodeficiency or other debilitating disease (10, 31).
A similar notion can be developed with *S. commune* (4, 7, 17, 23). Being abundant in the environment, basidiospores of mushrooms and shelf fungi are easily inhaled and with their small size deposited in pulmonary alveoli. Local or systemic impaired function of alveolar macrophages may allow settlement of a fungal thallus. Therefore, the shelf fungi are not to be categorized as primary pathogens. *S. commune*, for example, is a psychrophilic fungus of which fruiting body formation is stimulated by low winter temperatures. Possibly the shelf fungi, naturally being involved in lignin degradation, have only limited virulence, whereas with the ruderal mushrooms *C. cinereus* and *V. volvacea* a higher degree of invasive potential may be expected. This is evident from a high case fatality (73%) in invasive infections due to *C. cinereus* reported in 8 of the 11 patients of hematological malignancies described in literature so far (1, 9, 21, 24, 33, 37). Also, a solitary fatal case of *V. volvacea* in a patient following double umbilical cord blood transplantation has been reported (30).

Two of the patients were administered itraconazole which showed low *in vitro* MICs (0.06-0.5 µg/ml), but the outcome could not be assessed. The cases presented herein highlight the clinical relevance of this mold in pulmonary diseases. Cases 1, 2 and 4 demonstrate the ability of the mold *C. lacerata* to produce bronchopneumonia, which was fatal in case 2 and probably in case 1 but the patient could not be followed up and telephonic inquiry revealed that he died. Case 3 however, shows that this mold, similar to other basidiomycetes, can exist in the respiratory tract as a commensal without causing any obvious disease. This is for the first time that *C. lacerata* has been reported from clinical specimens. Environmental strains of the fungus have been isolated from white-rotted trees in Japan and Korea (18, 34). It is likely, that the first two cases, being farmers, acquired this infection through exposure to airborne spores at the workplace. It may be emphasized that, though the clinical significance of the isolation of basidiomycetes in healthy patients is probably negligible, a pathological
role in debilitated patients cannot be excluded. This communication adds another basidiomycete to the list of fungi associated with humans.

ACKNOWLEDGEMENTS:
This work was carried out, in part, with financial assistance from the Department of Biotechnology (ref. No. BT/39/NE/TBP/2010), New Delhi.

CONFLICT OF INTEREST: J.F.M received grants from Astellas, Merck, Pfizer, Schering-Plough, Gilead and Janssen Pharmaceuticals. He has been a consultant to Basilea and Merck and received speaker’s fees from Merck, Pfizer, Schering-Plough, Gilead and Janssen Pharmaceutica. All other authors: no potential conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Figure 1: White rotting mold growth of *Ceriporia lacerata* (VPCI 1921/11; CBS 133711) on (a) *Syzygium cumini* bark piece (size: 3-4 cm x 0.5-1 cm) and (b) Tomato (*Solanum lycopersicum*) twigs (3-4 cm long), inoculated on Potato dextrose agar (PDA) incubated at 28°C in light for 4 weeks (c) Lactophenol cotton blue mount of the mold (*C. lacerata*, VPCI 1921/11, CBS 133711) growing on wooden bark of *S. cumini* (inoculated on PDA) showing spicules (arrow) along hyphae after 3 weeks of incubation at x 400 magnification (d) Lactophenol cotton blue mount of the same mold growing on wooden bark inoculated on PDA showing ellipsoid basidiospores of 3.5-4.5 x 2.4-2.9 µm as seen after 4 weeks at x 400 magnification.