SPOROTHRIX SCHENCKII

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Sporothrix schenckii complex is a group of ubiquitous, eukaryotic, heterotrophic, thermally dimorphic fungal pathogens responsible for the development of noduloulcerative lesions in skin and subcutaneous tissue. The mold form of the fungi is commonly present in the environment (in soil, on plant material), whereas the yeast form is commonly seen in human & animal hosts.[1] It was initially assumed that only one species, *Sporothrix* schenckii, was involved in the infection, but later, molecular and phenotypical analysis of the *Sporothrix schenckii* complex determined that the group consisted of *Sporothrix schenckii sensu stricto, Sporothrix globosa, Sporothrix brasiliensis* and *Sporothrix luriei*, in addition to other cryptic species.[2] Following traumatic inoculation with either *Sporothrix*-contaminated thorns, less frequently by scratches from infected cats, and rarely, post inhalation of the fungal spores, the fungi would enter the host and cause subcutaneous tissue infection. This disease came to be called by many names – Sporotrichosis, Rose Gardner's disease, Rose Picker's disease, Schenck's disease, Rose Handler's disease, and Rose Thorn disease. [3,4] Floriculture, horticulture, wood exploitation, and mining were activities commonly associated with the infection. [5] *S. brasiliensis* was often associated with zoonotic infections transmitted by cats. [6]

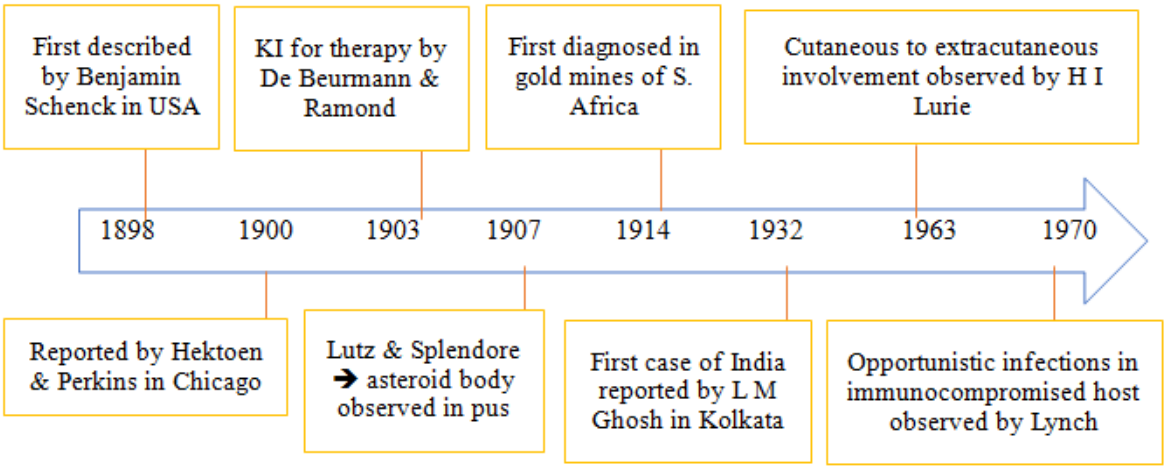
The classification of the fungi is as follows:

- Kingdom: Fungi
- Division: Ascomycota
- Class: Pyrenomycetes
- Order: Ophiostomatales
- Family: Ophiostomataceae
- Genus: *Sporothrix*
- Species: *schenckii* [7]

I. HISTORY OF SPOROTRICHOSIS

The first record of Sporotrichosis was documented by Benjamin Schenck in the United States in 1898, followed by the second case reported by Hektoen and Perkins in Chicago in 1900. In 1903, potassium iodide (KI) was established as a therapeutic agent against the infection by De Beurmann and Ramond. Lutz and Splendore reported the first natural animal infection case and later described asteroid body in pus discharging from the skin lesions of a *Sporothrix*-infected patient in 1907-08. [8]

The fungus was originally classified under the genus *Sporotrichum* by Smith, which was subsequently allotted to *Sporothrix* genus as *Sporothrix schenckii* in 1962. Lutz and Splendore described the yeast form of the fungus in 1947.[8] The first case of Sporotrichosis was diagnosed in gold mines of South Africa in 1914; whereas in India, the first case was recorded in 1932 by L M Ghosh. Sporotrichosis outbreaks were recorded in 1941-44 in South Africa, 1988 in the USA, 1995-97 in Peru, 2000-03 in Western Australia, and 1998 onwards in South-eastern Brazil. In 2008, *S. schenckii* var *luriei* was renamed as *S. luriei.* [5]



II. FUNGAL MORPHOLOGY

Sporothrix is a eukaryotic, heterotrophic fungus with chitinous cell wall and no sexual form or teleomorph. Thermal dimorphism is a character influencing the morphology of the fungus. [5]

In the environment and at 25°C, *Sporothrix schenckii* complex exists as a filamentous mold with 1-2µm wide hyaline, septate hyphae. Conidiogenous cells arise perpendicularly from the hyphae, terminating in clusters of single-celled, tear-shaped/clavate conidia. The conidia of *Sporothrix schenckii* have dark cell walls, distinguishing it from other species. [9,10] Inoculation onto Malt agar or Potato dextrose agar produces smooth, wrinkled, white to cream-colored colonies which develop black to brown color on further incubation. [11,12]

In the human or animal tissues, and at temperatures above 37°C, *Sporothrix* exists as a round or oval, unicellular budding yeast of 2-6 µm diameter that forms cream or tan-colored, smooth, colonies. [13] Inoculation of mycelia or conidia onto a nutritionally rich culture media, like Brain Heart Infusion (BHI) agar, and incubating the plate at 37°C mimics the infection in a patient and allows the conversion of the mold form to yeast form of the fungi. [14] Budding at the hyphal tips and along the hyphal wall gives rise to the yeast cells.[15]

The cell membrane of the fungi has invaginations which are more abundantly present in conidia than the yeast form, while hyphae have none. Outside the cell membrane, lies the rigid and complex cell wall. [16]. Components constituting the cell wall include chitin, peptidorhamnomannan, lipids, proteins, melanin granules, and glycopeptides. [17, 18, 19]

III. EPIDEMIOLOGY

Sporothrix schenckii complex are ubiquitous– they are naturally present in the environment as a saprophyte in the soil, animal excreta, and on dead and decaying vegetation, utilizing the organic matter available for its development. [11] Specific plants associated with the fungi are dead sphagnum moss, roses, and hay. [20,21] Sporotrichosis infections are commonly encountered in tropical and temperate areas, specifically in autumn and winter seasons, and endemically seen in Japan, India, Mexico, Brazil, China, Australia, etc. Sphagnum moss remains the main source of Sporotrichosis in the United States. [6]

Sporotrichosis infection can occur in patients irrespective of gender and age, but incidence is influenced by the occupation of the patients and the exposure to the fungus. The fungi are commonly transmitted from soil harboring mycelial phase of the fungi to the host via traumatic cutaneous inoculation like scratches, thorn pricks, etc. Among people who handle, own, or treat cats, the infection is transmitted following bites or scratches from the cat, or exposure to the nails, lesions, or habitat of the cat. Rats, squirrels, and armadillos are other animals that can be infected with the disease. [22, 23] Rarely, inhalation of conidia can also lead to Sporotrichosis. The disease has also been associated with outdoor leisure activities, such as gardening, floriculture, horticulture, mining, fishing, hunting, farming, etc. [12] Though human-to-human transmission is rare, the yeast form of *S. brasiliensis* can be transmitted between mammals and is often associated with zoonotic *Sporothrix* infections. [24] Other predisposing factors for the infection include diabetes mellitus, chronic alcohol abuse, cancer, and chronic treatment with steroids, etc. [4]

Central and South America, Southern USA, Africa, China, and South-East Asia have the largest number of cases. [6] *S. brasiliensis* infections are limited to Brazil, and *S. globosa* has been mainly reported in Asia. [8]

IV. CLINICAL MANIFESTATIONS OF *SPOROTHRIX SCHENCKII* COMPLEX

Sporotrichosis is initiated by traumatic inoculation of *Sporothrix* spores or mold filaments through the epidermis and subcutaneous tissues. Less frequently, inhalation can lead to the development of infection. The immunological status of the host, number of conidia or yeast cells in the inoculum, depth of the inoculation, strain pathogenicity, and thermal tolerance influence the clinical manifestations. [25]

1. **Virulence Factors:** The ability of the fungi to tolerate higher temperatures, that is, thermotolerance, contributes to the virulence of the organism. *Sporothrix* growing at 37°C has been isolated from lymphatic, disseminated, and extracutaneous lesions; whereas isolates tolerating 35°C temperature were associated with fixed cutaneous Sporotrichosis. [11]

   Melanin production has been observed to occur in both mycelial and yeast forms of the fungi. [26] This enhances fungal resistance to phagocytosis by macrophages and also makes the fungi highly invasive, promoting the formation of multifocal granulomas. [27, 28]

   Fibronectin adhesins on yeast cells' surface aid in the adherence of the fungi to host tissue and further in dissemination to various parts of the body. [29] Ergosterol peroxide helps

fungi to evade the reactive oxygen species inside the phagocytes. [30] Serine proteases and aspartic proteases produced by the fungi aid in tissue invasion. [6]

2. **Fungal Immunity:** Cell-mediated immune responses are crucial in control of the infection and granuloma formation. On exposure to the fungi, CD4+ T lymphocytes release IFN-γ, which in turn activates macrophages. TNF-α induces nitric oxide production in macrophages. [31, 32] Reactive oxygen species are produced by macrophages, monocytes & neutrophils. [33]

   Complement C3b binds to the yeast cell surface of *Sporothrix* in the host and promotes phagocytosis, whereas the membrane attack complex formed leads to cell lysis. Toll-like receptor-4 (TLR-4) activates the innate immune system and induces oxidative bursts against the fungi. [34, 35] Though both Th1 and Th2 responses are necessary for protection against *Sporothrix,* Th1 immune response plays a major role in eliminating the infection. [4,6]

V. TYPES OF SPOROTRICHOSIS

1. **Cutaneous Sporotrichosis:** Cutaneous sporotrichosis occurs post minor traumatic inoculation of the fungi into the skin. Upon entry to the host body, *Sporothrix* converts from mold form (hyphae and conidia) to yeast form, except in cases of zoonotic transmission from cats where yeast form is introduced on scratching. The incubation period is around 3 weeks. The lesions developing are either localised to the site of inoculation with a verrucous appearance (fixed sporotrichosis) or may extend along the regional lymphatic vessels in a "sporotrichoid pattern" from the site of inoculation up to the main lymphatic ganglion (lymphocutaneous sporotrichosis). Lymphocutaneous sporotrichosis commonly occurs in the host extremities. The papule formed develops into a pustule followed by a subcutaneous nodule. Ischemia beneath the lesion causes the lesion to turn into an ulcer and form pus. The granulomatous lesions or sporotrichoid chancre are limited to the epidermis, subcutaneous tissue, and lymphatics. Lymphedema and elephantiasis can occur in patients with multiple inoculation sites in the same area. Auto-resolution of the infection can occur in chronic cases. Fixed cutaneous sporotrichosis is also called the vegetative form of the disease and the lesions have verrucous shape and well-demarcated borders along with a covering of bloody crusts. Patients with fixed sporotrichosis have a good prognosis as the infection indicates a strong immune response.

2. **Disseminated Sporotrichosis:** The fungi can spread by the hematogenous route or direct inoculation to involve other sites like bones and joints. Lesions in osteoarticular infection are either small granulomas or large lytic lesions, often involving one joint in immunocompetent individuals. Systemic sporotrichosis is seen in rare cases in immunodeficient patients. [36] Sinuses, kidneys, retina, mouth pharynx, nose, genitals, meninges, and lungs are other sites reported to be infected by the fungi. Extracutaneous infections are associated with risk factors like alcoholism, AIDS, hematological malignancies, corticosteroid use, diabetes, cirrhosis, pregnancy, etc. [37]

3. **Pulmonary Sporotrichosis:** It is a rare form of sporotrichosis that occurs on inhalation of the *Sporothrix* conidia and is commonly an asymptomatic chronic disease. Condensation areas and miliary infiltrates are observed on radiological analysis. A

heavier fungal load inhalation or diminish in the host immune status leads to symptoms like pneumonia, moderate cough, and expectoration developing. Alcoholics and patients with severe underlying COPD (chronic obstructive pulmonary disease) are common cases where pulmonary sporotrichosis has been reported. [38]] In patients with acute, progressive infection, symptoms include weight loss, productive cough, and dyspnoea and radiological inspection shows hilar adenopathy with mediastinal widening

4. **Veterinary Sporotrichosis:** The infection occurs more commonly in cats than dogs and other animals. Zoonotic transmission occurs from infected cats and dogs. Lesions appear as ulcerative nodules on the head, nose, and ears with fistula development, pus formation, fever, and anorexia. Disseminated infection in cats affects the liver, lungs, spleen, kidneys, CNS, etc.

Differential Diagnosis of Sporotrichosis

Sporotrichosis	Differential Diagnosis
Cutaneous-lymphangitic sporotrichosis	cutaneous tuberculosis, syphilis, mycetoma, tularemia, chromoblastomycosis, pyogenic infections, tuberculoid leprosy, and infections caused by nontuberculosis mycobacteria
Fixed cutaneous sporotrichosis	verrucous tuberculosis, chromoblastomycosis, leishmaniasis, squamous carcinoma, impetigo, infections caused by nontuberculosis mycobacteria, and Orf disease
Disseminated cutaneous sporotrichosis	tuberculosis, syphilis, coccidioidomycosis, and infections caused by nontuberculosis mycobacteria
Pulmonary sporotrichosis	pneumonia, tuberculosis, aspergilloma
Extracutaneous sporotrichosis	histoplasmosis, bacterial infections, and cryptococcosis

VI. DIAGNOSIS OF SPOROTRICHOSIS

While serological, histopathological, and molecular approaches have been developed to diagnose the Sporotrichosis, fungal culture remains the gold standard method.

1. **Specimen:** Samples collected include tissue biopsy specimens and exudate from lesions. Blood, sputum, urine, CSF, and synovial fluids are collected based on the organ affected in disseminated sporotrichosis.

2. **Microscopy:** The fungal burden of yeast cells is scarce in human infections as compared to infection in cats, hence mount preparation using 10-20% KOH (potassium hydroxide) reveals budding yeast cells of 2-6 µm diameter. Gram staining of the sample shows Gram-positive budding yeast cells. Giemsa staining, Fluorescent-antibody staining, and Calcofluor white staining are other recommended staining methods for *Sporothrix* detection. Cigar-shaped elongated yeast cells of the size 2-3 µm x 3-10 µm are characteristic of the species. Pus collected from the lesion can be directly observed without 10% KOH to visualize asteroid bodies (clumps of yeast-like cells resembling crowns). [13]

The fungal mycelial form appears as thin, hyaline, septate, 1-3 μm wide hyphae with branching. Microconidia shape varies – ovoid or pyriform conidia arise from denticles on 10-30 μm long conidiophores perpendicular to the hyphae, resembling "daisy flower"; and microaleurioconidia or raduloconidia (sessile conidia) arise from hyphae. The conidia thicken their walls, group together, and produce melanin on detachment from the hyphae.

Tissue samples can be stained with histopathological stains like Hematoxylin & Eosin (H&E), Gomori Methenamine Silver (GMS), and Periodic Acid-Schiff stain (PAS). Fontana Masson stain is not recommended. In the skin and subcutaneous tissue, the *Sporothrix schenckii* complex causes suppurative and granulomatous inflammatory reactions. Microabscesses, fibrosis, hyperkeratosis, parakeratosis, and pseudoepitheliomatous hyperplasia may also be seen in the tissues. In addition, the localised immunological response of the host to *Sporothrix* antigens appears as a central eosinophilic focus (budding asteroid bodies) surrounded by radiating homogenous, eosinophilic, refractile clublike material, and this is called the Splendore-Hoeppli reaction. [13] In the asteroid bodies, the yeast cells remain viable, protected from the immune response of the host [39]

3. **Fungal Culture:** The specimen is inoculated onto Sabouraud's dextrose agar (SDA) with Chloramphenicol and Cycloheximide (actidione), Yeast extract agar. The SDA tubes are incubated at 25°C for 5-7 days. To demonstrate the thermal dimorphism property of the fungi, enriched media like Brain-Heart infusion (BHI) agar, chocolate agar, or blood agar plates are inoculated with the sample and incubated at 37°C for 5-7 days. *Sporothrix* can tolerate up to 0.25% cycloheximide and grows optimally at a temperature range of 28°C - 30°C, with the growth hampering above 40°C. [2] The mold form of *Sporothrix* grows optimally at a pH range of 3.0- 11.5, whereas the yeast form requires a pH of 3.0-8.5. The yeast phase is more osmotolerant and halophilic (11% NaCl) than the mold form (7% NaCl) of the fungus. [40] Humidity must be maintained >92%. [11]

   The mycelial form growth appears as membranous, radial colonies with whitish or beige-brown color. Subsequently, aerial mycelium develops with coremium. Growth of the yeast form on the enriched media incubated at 37°C appears as yellowish to tan color, creamy colonies containing enlarged yeast cells with fragmented mycelia. [14] Exposure to 5% CO_2 also promotes mold-to-yeast conversion.

   Slide culture can be performed to appreciate the production of dematiaceous conidia on Potato Dextrose agar (PDA) or Cornmeal agar (CMA). [41]. Lactophenol cotton blue (LPCB) staining is carried out to observe hyaline, ovoid conidia arranged in clusters on conidiophore perpendicular to thin, septate, hyaline hyphae.

   The fungi have the capacity to assimilate glucose, fructose, mannose, and cellobiose, with variable results for sucrose, arabinose, starch, raffinose, starch, and ribitol. [40] *S. schenckii* cannot ferment carbohydrates. [9] The fungi have the capacity to synthesize melanin but the amount varies depending on the carbohydrate concentration available to the fungi. [42] Urease enzyme is produced by fungi. [40]

4. **Serological Tests:** Original antibody detection tests included Precipitation tests, Agglutination tests, Double immunodiffusion tests, and Immunoelectrophoresis. [43]

Immunoenzymatic assays and enzyme immunoassays using mold form exoantigens are recent developments in the serological testing for Sporotrichosis.

5. **Molecular Detection Methods:** PCR can also be carried out for rapid diagnosis of Sporotrichosis, especially where samples have low fungal burden. [6} The genes targeted are internal transcriber space in the rRNA gene, chitin synthase gene, 26s rRNA gene fragment, DNA topoisomerase II gene, etc.

6. **Sporotrichin Skin Test:** It is a skin test to detect delayed-type hypersensitivity reaction to Sporotrichin antigen. The reaction is also positive in case of past infection with *Sporothrix*. It is carried out as a diagnostic test but is valuable for epidemiological purposes where soil samples are also collected for fungal isolation from the specified area. The test unfortunately lacks standardisation. [44]

7. **Treatment:** The drug of choice is Itraconazole – 200mg/day for 3-6 months or in intermittent dosing of 400mg/day for 1 week followed by no treatment for subsequent 3 weeks[45]. In the case of children infected with *Sporothrix*, 125-250mg/day of Terbinafine is recommended [5]. Oral administration of Potassium iodide (KI) (20g in 300mL of water) is an alternative treatment followed in low-income countries. Saturated suspension of KI can be given orally instead, beginning with 5 drops thrice a day and gradually increasing the number of drops administered to 40-50 drops thrice a day. KI treatment is given for 3-5 months [46]. As *S. schenckii* complex organisms cannot grow at temperatures above 40°C, a warm bath (45°C) at the site of the lesion for 15-20 minutes twice or thrice a day can be additionally given to the patient to limit dissemination of infection to uninfected tissues. Lipid formulations of Amphotericin B (3-5mg/kg/day) are recommended for disseminated sporotrichosis and in pregnant female patients [47].

Infection by *Sporothrix* can be prevented by using protective covering while gardening and early identification and treatment of infected cats [6].

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