MYCOLOGY

DR. O. E. OJO

DIAGNOSIS OF FUNGAL DISEASES

- Diagnostic cultural procedures for fungi should be carried out in the biosafety cabinet.
- Fungi produce spores which are easily get carried by air and can be dispersed in the environment.
- Dispersal of pathogenic fungi in air is of public health concern.
- Clinical signs and history are essential for making a tentative diagnosis.
- Specimen collection: samples are collected based on observed clinical sings and pathological lesions.
- Superficial nycosis (e.g. dermatophytosis): hair sample and skin scrapings
- Sub-cutaneous and systemic mycoses: biopsy, exudates, aspirates and tissue at postmortem
- Swab samples are inadequate for fungal diagnosis
- Stringent transportation conditions are not required for samples for fungal identification. Do not freeze. Most fungal agents can be recovered from samples in transit for about 14 days
- Penicillin-streptomycin or chloramphenicol is added to combat bacterial contaminants

Diagnostic procedures:

Microscopy:

Direct microscopic examination of wet preparation: skin scrapings and hair are mounted on microscope slide, treated with few drops of 10% KOH for about 2-3 hours to clear the specimen of debris and cover with cover slip. This is examined under the microscope at X40 magnification

to view arthropores or hyphae. Phase contrast microscopy helps in direct microscopy. Direct microscopy is fast, does not need staining and provides clear visualization.

Stained preparations:

- Methylene blue and Gram's methods can be used to demonstrate Yeast cells in samples.
- Moulds are demonstrable by lactophenol cotton blue.
- Wood's lamp: spores of *Microsporum canis* fluoresce when infected hairs are examined by Wood's lamp
- Silver nitrate/methenamine silver impregnation: for demonstration of fungal elements in tissue by impregnation
- Periodic acid Schiff: preferred for the demonstration of fungal elements in histological section. Fungi stain red
- Giemsa and Wright's stain: for biopsy and tissue from post mortem
- Gomori: for biopsy and tissue from post mortem. Fungi stain deep black while the tissue stains green
- Nigrosin and Indian ink: for demonstration of the capsule of *Cryptococcus neoformans*. When stained, budding cells with wide capsule is revealed.

Fungal culture:

Culture media:

- Sabouraud dextrose agar: primarily for isolation of dermatophytes from cutaneous samples or yeasts (*Candida spp*) from clinical samples. This medium has acidic pH (5.5) which inhibits the growth of most bacteria.
- Czepak's agar: for colonial study of *Aspergillus spp*

- Sabhi agar/inhibitory mould agar: recommended for improved recovery of fastidious, slow-growing fungi.
- Potato dextrose agar: for isolation of mould and yeast
- Selective culture: addition of chloramphenicol (16μg/ml), penicillin (20 I.U/ml), streptomycin (40 U/ml), gentamicin (5 μg/ml) and cycloheximide/actidione (0.5mg/ml) increases selectivity by inhibiting contaminations by bacteria and fast growing fungi (e.g. Zygomycetes)
- Enriched media: Brain-heart infusion agar is supplemented with 5% blood. This is used to stimulate the growth of yeast phase of dimorphic fungi. Incubation is at 37 ^{O}C.

Culture procedures:

Fungi are cultured in tubes or in plates (Petri dishes). Clinical samples are inoculated directly onto prepared medium and incubated aerobically.

Tube: agar media are poured and allowed to solidify in slanting position to prevent dehydration during prolonged incubation. The tubes are plugged with cotton wool or covered with screw cap. The cap should not be screwed tightly after inoculation to allow access to oxygen.

Plate: this provides large surface area and good hydration for fungal growth. It allows easy examination and subculture from the plate. The plate is taped round to prevent escape and dispersal of fungal spores.

Incubation condition:

Fungi are cultured aerobically. A flat pan containing water is placed under the incubator to provide humidity.

Conditions for Fungal incubation

Fungi	Temperature (^{O}C)	Duration of Incubation

Dermatophytes	25	2 to 4 weeks
Aspergillus spp	37	1 to 4 days
Pathogenic yeast	37	1 to 4 days
Dimorphic fungi (mould)	25	1 to 4 weeks
Dimorphic fungi (yeast)	37	1 to 4 weeks
Zygomycetes (fast growing fungi)	37	1 to 4 days

Fungal growth identification:

Criteria for identification:

- Condition of growth (temperature and duration of incubation)
- Colonial characteristics: Size and appearance, colour of obverse and reverse sides, surface elevation or depression
- Microscopy: examination of sporing head for conidia, presence or absence of septa, colour =of hyphae (hyaline/colourless, dematiaceous/pigmented), presence of special hyphal structure such as being racquet-shaped and spiral hyphae
- Biochemical reaction of yeasts
- Immunological test/serology: gel diffusion test, complement fixation test, agglutination reactions, delayed hypersensitivity test (purified protein derivatives)
- Molecular identification: using polymerase chain reaction, dot blot hybridization, specific nucleic acid probes and other molecular characterization

CANDIDA SPECIES

Candida spp occur worldwide and can be found as commensals on plant as well as in the digestive and urogenital tracts of humans and animals. There are more than 200 species in the genus *Candida.* However, *Candida albicans* is the species most often implicated in human and animal disease. Other species are *C. tropicalis, C. krusei, C, stellatoides, C. paratropicalis*. *Candida albicans* does not have a sexual stage. It grows aerobically at 37 ^{O}C on a wide range of media including Sabouraud dextrose agar (SDA). Colonies are composed of budding oval cells about 5 by 8µm. *Candida albicans* may exhibit polymorphism (pseudohyphae and true hyphae) in animal tissues. On some culture media under certain conditions, it may also form thick-walled resting cells referred to as chlamysospores (chlamydoconidia).

Clinical conditions

Candida spp are associated with opportunistic infections. These diseases occur sporadically and are associated with immunosupression resulting from malnutrition, metabolic disorders (diabetes mellitus), pregnancy, corticosteroid therapy, stress and elimination of competing bacterial flora subsequent to prolonged antimicrobial use.

Clinical conditions associated with *Candida albicans*

Mycotic stomatitis in puppies, kittens and foals; gastro-oesophageal ulcers in pigs, foals and calves; rumenitis in calves; enteritis and dermatitis in dogs; thrush of the oesophagus and crop in chickens; cloacal and vent infections in geese and turkeys; reduced fertility, abortion and mastitis in cattle; pyometra in horses; urocystitis and pyothorax in cats; ocular lesions in cats and horses and disseminated/generalized infection in dogs, cats, swine and cattle.

Diagnosis:

- Clinical samples: biopsy specimen, tissues from postmortem, sputum, exudates and discharges, skin scrapings, milk, faeces.

- Tissue section stained with methenamine silver impregnation or Periodic acid Schiff to demonstrate budding yeast cells and hyphae
- Isolation of *C. albicans* following aerobic culture of specimen of SDA with or without cyclohexide supplement. Culture is incubated at 37 ^{O}C for 2 to 5 days.

Identification criteria

- Colonial appearance: colonies of most species of *Candida* are similar. The colonies are whitish, slimy and convex. They are about 4 to 5 mm in diameter after 3 days of incubation.
- Only *C. albicans* grows in the presence of cycloheximide, other species are inhibited with cycloheximide.
- *Candida albicans* produces germ tubes within 2 hours when incubated in serum at 37 ^{O}C. Germ tubes are round yeast cell with slender cylindrical protrusions produced by *Candida albicans* within 2 hours when incubated in serum at 37 ^{O}C.
- *Candida albicans* produces chlamydospores in submerged culture on corn meal agar. Chlamydospores are thick-walled resting cells formed from pseudohyphae in submerged colonies growing in cornemeal agar.
- Microscopy: Gram staining reveals Gram positive organism. Colonies of *Candida* yield characteristic budding yeast cells.
- Biochemical profiles: carbohydrate assimilation and fermentation tests for the definitive identification of *Candida species*.

Treatment:

Nystatin and Miconazole are used for the treatment of localized infection without internal involvement. Amphotericin B and 5-fluorocytosine used for systemic treatment.

CRYPTOCOCCUS SPECIES

Although the genus *Cryptococcus* contains about 37 species, only *C. neoformans* produces clinical infection. *Cryptococcus neoformans* is a round to oval yeast of about 3.5 to 8.0 μm in diameter. *Cryptococcus species* exist only in the yeast form (there is no mould/mycelia form). A budding daughter cell is usually seen on a narrow neck joined to the mother cell. They are aerobic, non-fermentative organisms which form mucoid colonies on a variety of media including SDA. When freshly recovered directly from infected animal host, the yeast possesses thick mucopolysaccharide capsule demonstrable by Indian ink. Four serotypes (A, B, C and D) of *C. neoformans* are recognized. Classification into serotypes is based on the type of capsular antigen possessed. Serotypes A and D are designated as *C. neoformans var neoformans* while serotypes B and C are termed *C. neoformans* var *gatti*.

Cryptococcus neoformans can be recovered from the droppings of pigeons and other birds. Soil enriched by such the droppings is also a good source of the organism. *Cryptococcus neoformans* has affinity for creatinine present in the droppings. Pigeons can excrete *C. neoformans* for several months without showing signs of clinical infection.

Clinical infection: *Cryptococcus neoformans* produces opportunistic infection in animal host. Infection is transmitted by inhalation of *C. neoformans* in dust. The organism is associated with the following clinical conditions:

- Cattle: mastitis and nasal groanulomas
- Horses: nasal granulomas, sinusitis, pneumonia, meningoencephalitis, abortion and cutaneous lesions
- Dogs: generalized infection with prominent neural and ocular signs
- Cats: respiratory, neural, ocular and cutaneous diseaases
- Humans: respiratory (tuberculosis-like) lesions and meningoencephalitis

Microbiological diagnosis:

Note: exercise caution in handling samples from suspected cases because of the risk of zoonotic transmission of *Cryptococcus neoformans* to humans.

- Clinical samples for diagnosis: exudates, cerebrospinal fluid, biopsy specimens and tissues from postmortem.
- India ink preparation of sample will reveal budding yeast with prominent capsule
- In tissue section, capsule can be demonstrated by Mayer's mucicarmine method.
- Melanin can be detected in cell wall of *C. neoformans* by the Fontana-Masson technique
- Isolation of the organism from clinical sample is achieved by inoculation onto SDA with incorporation of chloramphenicol. Cycloheximide should not be added. Inoculated medium is incubated at 37^{O}C for up to 2 weeks. Ability to grow at 37^{O}C distinguishes *C. neoformans* from other *Cryptococcus* species. In addition, *C. neoformans* produces brown colonies on birdseed agar.
- Identification criteria: Mucoid colonies, presence of capsule and urease activity
- Latex agglutination test for the detection of soluble capsular material of *C. neoformans* within 3 weeks of infection in CSF, urine and serum

Treatment: Amphotericin B and Flucytosine

SPOROTHRIX SCHENCKII

Sporothrix schenckii is widely distributed in the environment where it grows as a mould producing conidiophores and slender hyphae of about 1 to 2 μm in diameter. The organism causes sporadic infection in horses, cats, dogs and humans. *Sporothrix schenckii* is present in many parts of the world especially in the tropical and subtropical regions. It grows as a

saprophyte on dead and senescent vegetations such as rose thorns, timbers, hays, straws and mosses.

Clinical infections: *Sporothrix schenckii causes* chronic cutaneous and lymphocutaneous sporothricosis. The disease may become generalized and disseminated all over the body. Disseminated infection is common in immunocompromised patients. Sporadic cases have been reported in horses, cats, dogs, cattle, goats, pigs and humans. The organism enters the body through abrasion on the skin especially at the extremities (limbs). Nodular lesions develop on the lymph nodes and along the lymphatics. The nodules rupture leaving ulcers and exudates.

Diagnosis:

- Clinical samples: exudates, scrapings from ulcers, aspirates and tissues from affected lymph nodes
- Direct microscopy: examination of methylene blue-stained smear of exudates from lesions in affected cats reveals large number of yeast cells. These are sparse in exudates from other animals.
- Gram staining: smear made from pus reveals Gram positive budding yeast cells
- Histopathological examination of tissue section stained by PAS or methenamine silver impregnation may reveal yeast cells.
- Fluorescent antibody or immunoperoxidase technique applied to tissue section permit specific identification of yeast cells
- Fungal isolation:

  Mould form: Samples are inoculated on SDA at 25 ^{O}C to produce mould colonies. The colonies which develop rapidly are white becoming black or brown, wrinkled and leathery. Microscopic examination of the mould colonies reveals pear-shaped conidia borne in a rosette pattern on slender conidiophores. In old cultures, conidia form singly on hyphae.

Yeast form: to demonstrate the yeast form of the organism, samples are inoculated onto brain heart infusion agar containing 5% blood and incubated at 35-37 ^{O}C. Cream colour to tan yeast colonies develop within 3 weeks. At microscopy, cigar-shaped yeast cells of about 2-3 μm by 3-5 μm in size are observed.

Treatment: administration of inorganic iodide (potassium iodide or sodium iodide) in feed. Treatment should continue for 30 days after complete recovery. Ketoconazole may be given along with inorganic iodide in intractable cases. Intravenous administration of amphotericin B could also be considered.