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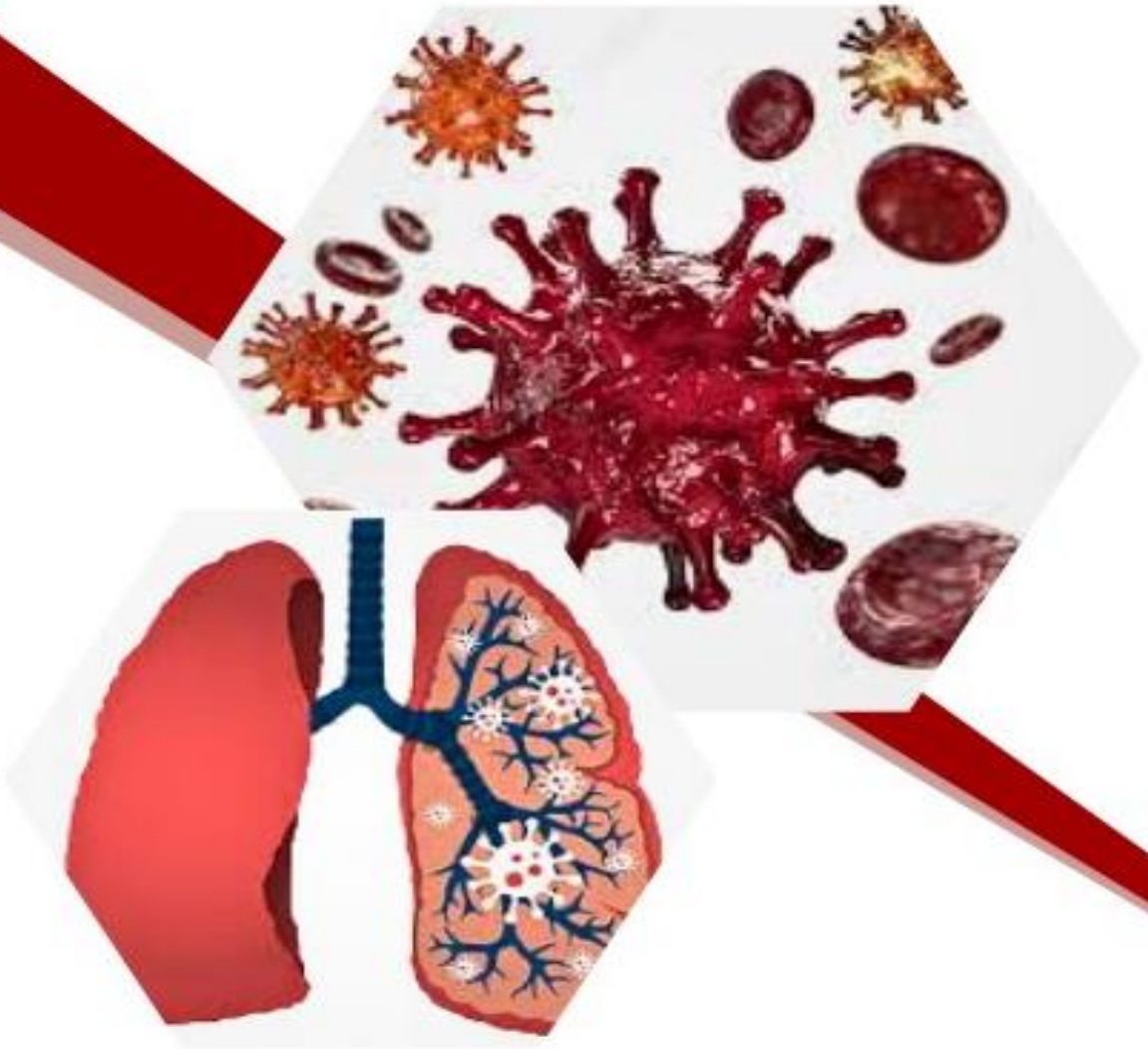
RS MICROBIOLOGY



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Atypical Pneumonia

Atypical pneumonia is caused by three bacterial pathogens:

1. **Mycoplasma** pneumoniae, which lacks a cell wall.
2. **Chlamydia** pneumoniae and Chlamydia psittaci, both of which are gram-negative bacteria.
3. **Legionella** pneumophila, also a gram-negative bacterium.

Atypical pneumonia is different from pneumonia caused by Streptococcus, which is considered typical pneumonia. The three bacteria mentioned earlier typically cause **milder forms of pneumonia**, characterized by a slow and gradual development of symptoms, unlike other forms of pneumonia that can develop more quickly.

Patents are still able to walk, function, and carry out daily activities without realizing they have pneumonia, hence the name **"Walking Pneumonia".** Another characteristic that distinguishes it from typical pneumonia is that, although it is generally less severe, **its early symptoms are more severe than those of typical pneumonia**. Additionally, these **bacteria do not respond to antibiotics commonly prescribed for streptococcal pneumonia (important)**, such as penicillin and cephalosporins.

It is difficult to diagnose these specific causes, so it is unknown what percentage they contribute to all cases of pneumonia. However, books state that 10% - 20% of community-acquired pneumonia cases are caused by Chlamydia pneumonia, Mycoplasma pneumonia, and Legionella pneumophila.

Mycoplasma pneumoniae and Chlamydia pneumoniae can **be transmitted from person to person** through droplet nuclei, but they are not highly contagious. Additionally, Mycoplasma and Chlamydia **can be** part of the normal flora, but the percentage of healthy carriers is not high.

In contrast, **infections caused by Legionella do not involve person-to-person transmission**. Individuals acquire it by breathing in aerosols containing Legionella, which typically contaminate water systems and **air conditioning systems**. Legionella can survive in these environments by forming biofilms or by residing inside amoeba.

1-Mycoplasma pneumoniae

The smallest size bacteria, Mycoplasma, have a distinguished feature of **lacking a cell wall**. As a result**, diagnosis using gram stain is invalid**, and they do not respond to penicillin and cephalosporins. Although there may be intracellular events in their life cycle, Mycoplasma are generally **considered extracellular pathogens.**

Members of Mycoplasma that cause human diseases:

1. Mycoplasma pneumoniae
2. Mycoplasma genitalium, which is subdivided into:

   a) Mycoplasma hominis (genitourinary tract infection)

   b) Ureaplasma parvum (genitourinary tract infection)

   c) Ureaplasma urealyticum.

- They have lipid bilayer membrane, **aerobic growth** and various Mycoplasma species that are associated with disease in humans, animals, and birds.
- M. pneumoniae is spread from **person to person through droplets**, but **it is not highly infectious.**
- Common symptoms of M. pneumoniae infection include low fever, dry cough, anemia, rashes, and neurological syndromes such as meningitis and encephalitis. These symptoms can develop over a few days to weeks.
- Infection caused by M. pneumoniae can range from acute/subacute pharyngitis **(often subclinical)** to bronchitis or a milder form of pneumonia.
- **Common syndromes associated with M. pneumoniae** usually develop 2-3 weeks after the initial infection and include **anemia** (hemolytic anemia) with production of cold agglutinins (auto-antibodies against antigens on red blood cells), as well as **neurological syndromes** such as meningitis, encephalitis, and brain abscesses.
- It is unknown whether the neurological syndromes are a direct result of mycoplasma extending to the central nervous system or an autoimmune response towards M. pneumoniae.
- if they were part of normal flora, they colonize respiratory and genitourinary mucosa.
- Common infections in the fall-winter season **mostly affect older children and young adults**. Unlike Chlamydia, which affects school-age children (Important).
- Severe forms of M pneumonia have been described in all age groups

Lab diagnosis and treatment

Lab diagnosis of Mycoplasma is **difficult** because they are common contaminants. However, special culture medium **(FRIIS Medium) and PCR** tests have been recently developed to detect Mycoplasma in pleural fluid or blood. The serological Cold-Agglutination test, which was previously used, is no longer recommended because it can detect infections other than Mycoplasma pneumoniae. Instead, serological tests are used to look for antibodies against Mycoplasma.

For treatment, **fluoroquinolones** such as levofloxacin and moxifloxacin, as well as **macrolides** like azithromycin, are commonly prescribed. It is important to note that there is currently no vaccine available for Mycoplasma.

Chlamydia species.

Chlamydia attaches to human mucosal membranes, allowing them to become **part of the normal flora**. **Person-to-person transmission is possible** but to a lesser extent than Mycoplasma. One of the reasons for this is that Chlamydia has two forms in its life cycle:

1) elementary body - the infectious form.

2) reticulate body - the metabolically active form.

The elementary body cannot survive outside the body in dry conditions, so **prolonged close contact is required for transmission**, particularly for Chlamydia pneumoniae. **It's important to note that** **Chlamydia psittaci is a zoonotic infection transmitted from Psittacine birds, such as parrots.**

They are Obligate intracellular organisms, intracytoplasmic inclusions, rapidly killed outside the body, dryness and high temperatures above 4°C

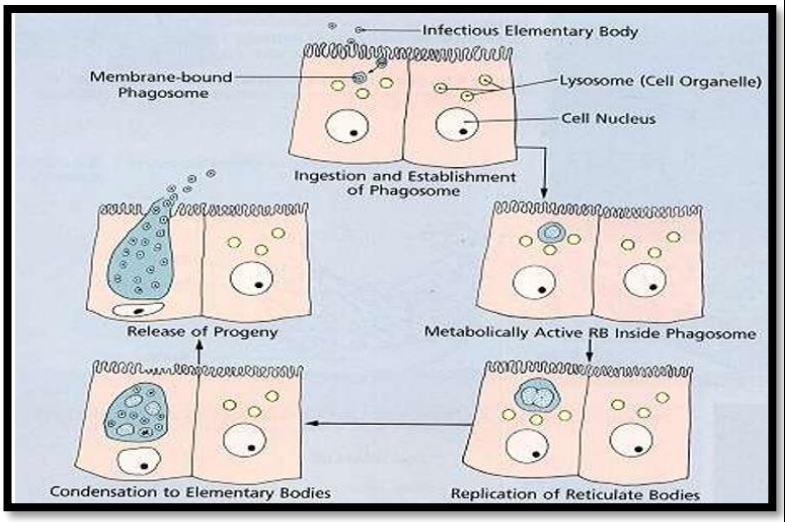
Chlamydia trachomatis, specifically serotypes C and K, is a common cause of sexually transmitted diseases (STDs). It can also lead to nonspecific urethritis and can be transmitted from mother to newborn babies through maternal fluids. atypical pneumonia, eye infection, **ophthalmia neonatorum**

Chlamydia trachomatis serotypes A and C are responsible for causing **Trachoma**, which is the leading infectious cause of preventable blindness in developing countries. Trachoma can result in conjunctival scarring, damage to the eyelids and cornea, and ultimately, blindness.

Additionally, approximately half of all newborns with Chlamydial pneumonia develop inclusion conjunctivitis. This condition presents with mild to severe symptoms including redness, swollen eyelids, inflammation, and a thick discharge from the eyes, typically occurring 1-2 weeks after birth.

It is important to note that while atypical pneumonia caused by Chlamydia is primarily associated with pneumoniae and psittaci, Chlamydia trachomatis can also cause atypical pneumonia, although its main effects are related to STDs and trachoma.

Chlamydia Life Cycle



Infectious elementary bodies attach to the host mucosa and promote their entry. They then enter the cytoplasm phagosome. **Inside the phagosome, the elementary bodies differentiate into reticulate bodies**, which are metabolically active but **noninfectious**. The reticulate bodies divide through binary fission. After division, the reticulate bodies reorganize themselves to form new elementary bodies. These newly formed reticulate bodies can leave the cell to infect adjacent cells or can be released from the body through droplet nuclei. They are infectious if there was **close prolonged contact**. However, they do not remain infectious for a long period of time because elementary bodies cannot withstand environmental conditions. This is why Chlamydia is not highly contagious.

Chlamydophila Pneumonia

C. pneumoniae is primarily transmitted through droplets and commonly **affects infants, children (especially school-age children)**, and adults. In infants and children, the infection often develops gradually over several weeks, presenting with mild respiratory symptoms, a prolonged dry and irritating cough, nasal congestion, and sometimes fever. Blood sepsis is rare in these cases.

In adults, C. pneumoniae infections are often **asymptomatic or mild**, with symptoms that may include sore throat (pharyngitis), headache, fever, and a dry cough.

Diagnosis and treatment

To diagnose C. pneumoniae, sputum and throat-nasal swab samples can be collected and subjected to gram staining (C. pneumoniae is gram-negative). Other diagnostic methods include ELISA for specific antibodies, MaCoy Cell Culture, and **Micro immunofluorescence (MIF), which is currently the most sensitive test for Chlamydia.**

Treatment for C. pneumoniae infections typically involves **tetracyclines, macrolides and fluoroquinolones such as levofloxacin and moxifloxacin**. It is important to note that there is currently **no vaccine** available for C. pneumoniae.

Chlamydophila Psittaci

C. psittaci is a bacterium that causes zoonotic diseases. Human infection occurs through contact with birds such as parrots, **pigeons**, turkeys, and ducks. This can lead to a rare human disease called **psittacosis (ornithosis).** People who keep birds, like parrots, are more susceptible to getting Chlamydia psittaci. The bacteria can be inhaled from feathers, secretions, and droppings, leading to localized inflammation in the bronchi and lung tissues.

The signs and symptoms of psittacosis start as a mild flu-like illness, which can progress to atypical pneumonia. In most cases, **the illness resolves spontaneously** without complications. However, severe cases can occur, including fatal pneumonia, high fever, dry cough, and headache.

Diagnosis and treatment of psittacosis are similar to other Chlamydia infections. (Important)

In some cases, tetracyclines are contraindicated, so the treatment macrolides and fluoroquinolones.

Legionella pneumonphila

Legionella is a Gram-negative bacterium that is often found in water systems, air conditioning units, and bath tubs as biofilms or inside amoeba. It can be found in natural aquatic bodies and wet soil. Legionella is a facultative anaerobe and can grow in cold or hot water (4-80°C). It is transmitted through inhalation, typically via air conditioning systems or wet soil, and can cause outbreaks of diseases.

It is important to note that Legionella **cannot** be transmitted person-to-person. Therefore, if someone infected with Legionella kisses someone else, they will not transmit the bacteria to the other person. Legionella is an obligate intracellular bacterium, meaning that once it enters the body, it gets picked up by alveolar macrophages. Legionella is known to mistake macrophages for amoeba, allowing it to enter and replicate within the macrophages.

Infection with Legionella primarily affects the lungs, where the bacteria multiply intracellularly within the macrophages. Symptoms of Legionella infection include high fever, an incubation period of 2-10 days, nonproductive or productive dry cough, shortness of breath, chest pain, muscle aches, joint pain, diarrhea, **renal failure, and a higher mortality rate.**

Two clinical forms of Legionella, collectively known as legionellosis, include:

1. Legionnaire's disease: **This is the most severe form** and is characterized by pneumonia, gastrointestinal symptoms (hepatosplenomegaly), and delirium. It can affect multiple organs, including the kidneys, as Legionella bacteria reside inside macrophages and can access the entire reticuloendothelial system.

2. Pontiac fever: **This is the most common form (Important)** and is characterized by fever, throat pain, and flu-like illness. Unlike Legionnaire's disease, Pontiac fever does not cause pneumonia.

Both Legionnaire's disease and Pontiac fever are not contagious. Risk factors for legionellosis include heavy cigarette smoking, old age, underlying diseases such as renal failure, cancer, diabetes, or chronic obstructive pulmonary disease, suppressed immune systems, and corticosteroid use.

Diagnosis and treatment of legionellosis.

Diagnosis of legionellosis can be done through special culture media, blood/urine specimens, or throat swabs for detecting specific antibodies or antigens using PCR or ELISA.

Treatment options for legionellosis include macrolides (such as azithromycin), levofloxacin, and moxifloxacin. However, there is currently no vaccine available for prevention.

Opportunistic Mycoses

There are opportunistic fungal infections and endemic systemic mycotic infections. The difference between them is those opportunistic fungal infections, also known as opportunistic mycoses, **have a very low pathogenic value**. They usually **affect immunocompromised individuals** rather than immunocompetent individuals, which is why they are called "opportunistic." On the other hand, systemic endemic mycotic infections can affect both healthy individuals (with 90-95% resolving spontaneously) and immunocompromised individuals, where they can lead to serious complications.

Opportunistic mycoses are caused by globally distributed fungi that can either be part of the human microbiota, such as **Candida species** (indigenous), or environmental yeasts and molds (exogenous). These infections can range from superficial skin or mucous membrane infections to systemic involvement of multiple organs.

Patients at risk of opportunistic mycoses include those with hematologic dyscrasias (e.g., leukemia, neutropenia), **patients with HIV/AIDS** with CD4 counts less than 100 cells/μL, as well as those treated with immunosuppressive drugs (e.g., corticosteroids), cytotoxic drugs, post-organ transplantation, cancer therapy, and even those who take broad-spectrum antibiotics.

Cryptococcus neoformans

Cryptococcus neoformans is the causative agent of cryptococcosis. It is a widespread **encapsulated yeast** that is commonly found in soil around pigeon roosts. The capsule of Cryptococcus is **anti-phagocytic**, allowing it to evade the immune system. The transmission of the fungus occurs through inhalation of spores from birds or pigeon droppings present in the soil.

Cryptococcosis is a common infection among individuals with compromised immune systems, such as those with AIDS, cancer, or diabetes. When the lungs are infected, symptoms such as cough, fever, and lung nodules may arise. If the infection disseminates to the meninges and brain, severe neurological disturbances and even death can occur. **Meningitis is the most recognized form of cryptococcosis (important)**, although the infection initially starts in the lungs. (So, you should know that **Cryptococcus is neurotropic**.)

Diagnosis of cryptococcus

- Microscopic: **India Ink is used for capsule stain** (important)(50-80% positive in CSF), which shows the capsule. However, it has a disadvantage of missing up to 50% of cases.

- Culture:
  - Bird seed agar
  - A **new test called Latex Particulate Agglutination test (LPA)** is used in Europe and USA, but not in Jordan. It is considered diagnostic for Cryptococcus.
  - Routine blood culture
- PCR

Aspergillosis: Diseases of the Genus Aspergillus

- very common **airborne soil fungus**, Aspergillus, is associated with two types of Aspergilloses:

  1) allergic aspergillosis.

  2) invasive aspergillosis.

- **Aspergillosis is most commonly caused by Aspergillus fumigatus**. Other members, such as Aspergillus parasiticus and Aspergillus flavus, are mainly involved in the production of aflatoxins.
- There are around 600 species of Aspergillus, with 8 species implicated in human disease. Among them, A. fumigatus is the most common.

- Aspergillosis poses a serious opportunistic threat to AIDS, leukemia, and transplant patients. Infection usually occurs when spores are inhaled and germinate in the lungs, forming **fungal balls known as** "aspergilloma." Invasive aspergillosis can also colonize sinuses, ear canals, eyelids, and conjunctiva.

- The mildest form of aspergillosis is rhinal allergy and bronchopulmonary allergy, clinically defined as asthma, with patients exhibiting high IgE levels. **Invasive aspergillosis in preformed cavities can lead to necrotic pneumonia** and infection of the brain (meningitis), heart (endocarditis), and other organs.
- **Treatment** options include surgery (debridement of aspergilloma, especially if cavitary lesions are present), Amphotericin B (intravenous), and nystatin.

Zygomycosis (Mucormycosis or rhinocerebral Mucormycosis)

Zygomycota are highly abundant saprophytic fungi that can be found in soil, water, organic debris, and food. The most commonly involved genera are **Rhizopus, Absidia, and Mucor**. Infection occurs through the inhalation of sporangiospores from these three species. Once inhaled, the **sporangiospores germinate in the nostrils** and become **highly invasive**, often reaching the brain. This is why the infection is referred to as "Rhinocerebral Mucormycosis". Normally harmless air contaminants, these fungi can invade the membranes of the nose, eyes, heart, and brain in **individuals with diabetes and malnutrition**, leading to severe consequences**. Diabetic patients with Rhinocerebral Mucormycosis typically present with diabetic ketoacidosis (DKA)**.

Diagnosis and treatment

The diagnosis is made by **direct smear** and by isolating the molds from respiratory secretions or **biopsy** specimens. Treatment involves controlling diabetes, **surgery**, and using antifungal medication such as amphotericin B. **The prognosis for this condition is generally very poor. (Important).**

Pneumocystis

Pneumocystis jirovecii (formerly known as Pneumocystis carinii) is the cause of a severe pneumonia in individuals with weakened immune systems, especially those with AIDS. This infection, known as pneumocystis pneumonia (PCP), is very common in AIDS patients. It can also cause interstitial plasma pneumonitis. Although it was previously considered a protozoan, ribotyping has shown that it is actually a fungus (Important…. It is a fungus).

A definitive diagnosis of pneumocystosis relies on identifying organisms with typical morphology in appropriate specimens, such as sputum or bronchoalveolar lavage (BAL). **The organism cannot be cultured ex vivo**, but it can be isolated from tissue biopsies and stained using a silver stain.

The treatment of choice for pneumocystosis is **TMP-SMX (Trimethoprim / Sulfamethoxazole**).

Endemic mycosis

Endemic mycosis is caused by a thermally **dimorphic fungus** that exhibits two morphological states: a filamentous form in the environment (at room temperature) and a yeast form (single cell) in the body (at body temperature**). Infections are typically initiated in the lungs when the respective conidia** (usually asexual) or spores (which can be sexual or asexual) are inhaled. Conidia and spores are the reproductive elements of fungi.

Endemic mycoses, including coccidioidomycosis, histoplasmosis, blastomycosis, and paracoccidioidomycosis, are geographically limited to specific areas of endemicity. Most infections are asymptomatic or mild and resolve without treatment. **However**, a small but significant number of patients (5%), particularly those who are immunocompromised or have other health issues, may develop pulmonary disease.

Histoplasmosis-1

Histoplasma capsulatum is a dimorphic fungus that exhibits conidia and yeast forms at body temperature, and hyphae and macroconidia in vitro culture. It is commonly found in soil enriched with bird excreta, making individuals who work with birds and poultry more susceptible to histoplasmosis. **It is the most prevalent fungal/mycotic infection worldwide**. Despite its name, "capsulatum," **it does not possess a capsule**. During staining, the cytoplasm shrinks, creating a hollow space between it and the cell wall, which was previously mistaken for a capsule. Histoplasma capsulatum is endemic in the southern United States and Australia, while it is less common in other countries, such as Africa**. In Africa, Histoplasma duboisii, not Histoplasma capsulatum**, is present.

The primary site of infection is typically the lungs, as the fungus is inhaled through dust containing microconidia, which are then phagocytosed by macrophages. Histoplasma capsulatum is an obligate intracellular parasite, causing a slight inflammatory reaction. Initially, **tuberculate macroconidia are inhaled**, which later convert into **microconidia** (the diagnostic form) inside the body. Most cases of histoplasmosis are **asymptomatic or subclinical**, resulting in a benign course. However, some individuals may experience a flulike syndrome that resolves spontaneously.

Few individuals, especially those who are **immunocompromised, may develop chronic progressive lung disease characterized by the formation of granulomas and fibrosis**. This disease can also involve any internal organ, leading to chronic cutaneous or systemic symptoms. In some cases, it can be fatal.

All infected individuals will test positive for the **histoplasmin skin test**, which detects antigens of Histoplasma in the skin or lungs. However, this test, along with other similar tests for endemic mycoses, **is not specific and can result in false positive results due to cross-reactivity with other endemic mycoses.** (Important) Nowadays, these tests are mainly used for epidemiological studies.

Since Histoplasma capsulatum **targets phagocytes**, it can gain access to the entire reticuloendothelial system, leading to manifestations such as **hepatosplenomegaly**.

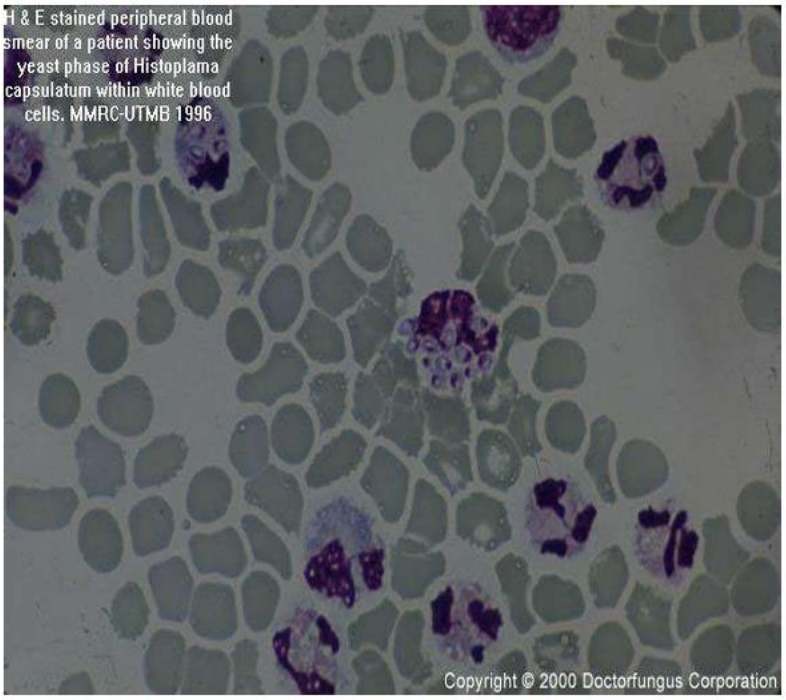


Figure 1-2 Histoplasma capsulatum in infected White Blood cells, notice it is obligate intracellular

Coccidioidomycosis & Blastomycosis

Coccidioides immitis causes coccidioidomycosis, also known as "Valley Fever," mainly in California. Blastomyces dermatitidis causes blastomycosis, a **soil-inhabiting dimorphic fungus**. Both are endemic in the southwestern U.S.A., northern Mexico, and various parts of South America.

The spores of Coccidioides immitis exist in **desert sand**, which is why they are called "Valley fever." The infectious spores of Coccidioides immitis are called "arthrospores," but in diagnosis, we look for "spherules" in tissue, where the spores are inside a sac called "endospores." When discussing fungi, these three terms (arthrospores, spherules, and endospores) **should always make you think of Coccidioides immitis**.

On the other hand, the spores of Blastomyces dermatitidis usually exist in decaying organic matter, such as decaying woods.

A characteristic feature of these two mycotic infections is the presence of mucocutaneous/skin manifestations in the form of subcutaneous nodules, such as erythema nodosum or erythema multiforme.

Respiratory infection, caused by inhalation of microconidia, often resolves rapidly, leaving the patient with **strong specific immunity to reinfection**. In some individuals, the disease may progress to a chronic pulmonary condition or a systemic disease involving the meninges, bones, joints, subcutaneous, and cutaneous tissues. The positive result of an antigen skin test is not significant in diagnosis.

Paracoccidioidomycosis

Paracoccidioides brasiliensis is a thermally dimorphic fungal agent that causes paracoccidioidomycosis, also known as South American blastomycosis. This fungal infection is limited to endemic regions in Central and South America. To differentiate it from Blastomyces dermatitidis, which is endemic in North America, we can observe certain characteristics. In Blastomyces dermatitidis, we would look for a single cell with a broad-based **single** bud, while in Paracoccidioides brasiliensis, we would find a single cell with broad-based **multiple** buds. This is in contrast to Cryptococcus neoformans, which has narrow-based buds.

The primary route of transmission is through inhalation of the corresponding spores. While most infected individuals remain asymptomatic, some may develop chronic progressive pulmonary disease or systemic disease involving multiple organs. P. brasiliensis is inhaled, and initial lesions typically occur in the lungs. After a period of dormancy that can last for decades, the pulmonary granulomas may become active, leading to chronic progressive pulmonary disease or dissemination.

Laboratory Diagnosis

The same approach is used in all fungal infections.

- Direct microscopy and culture should be performed on all specimens, including sputum, bronchial washings, CSF, pleural fluid, and tissue biopsies from various visceral organs.
- Wet mounts in 10% KOH with India ink can be used to observe ovoid-budding yeast cells.
- Gram stain smear can also be used.
- Microscopic smears processed with KOH should be observed for morphology, although this test requires expertise and other diagnostic techniques may be time-consuming.
- Cultures on Sabouraud dextrose agar should be maintained for one month at 25°C to detect fungal growth.
- Wet mounts can also be used for identification, observing hyphae-like conidiophores and spores, as well as the color of fungal growth.
- Serological tests have limited value, but antibodies can be looked for using immune diffusion and complement fixation tests. However, these tests are not suitable for diagnosing acute mycotic infections as they require the patient to develop antibodies over a two-week period.
- Detection of Histoplasma antigen in blood and urine is significant.
- Skin tests similar to tuberculosis skin tests were previously used but are no longer significant due to cross-reactivity and anergy in some individuals, leading to negative results even in the presence of endemic mycotic infections, which is a poor prognostic sign and may indicate the potential development of fulminant disease.