Acid fast staining of sputum is the initial test. Ziehl Neelsen.

For rapid screening purposes, auramine stain fluorescence microscopy is used after digestion of the specimens by treatments with NaOH and concentration by centrifugation, the natural is cultured on special media. Such as Lowenstein-jensen agar, for up 8 weeks. It will not grow on a blood agar plate. In liquid medium is preferred for isolation because the organisms grows more rapidly and reliably then it does on agar. If growth in the culture occurs, the organism can be identified

M. tuberculosis produce niacin, whereas almost no other mycobacteria do. It also produce catalase. Nucleic acid amplification test can be used to detect the presence of M. tuberculosis directly in clinical specimens are highly specific, such as sputum. Tests are available that detect either the ribosomal RNA or the DNA of the organism. These test are highly specific, but their sensitivity varies. Because during resistance especially to isoniazid is a problem, susceptibility tests should be performed. The organisms grows very slowly, and the susceptibility tests usually takes several weeks, which is too long to guide the initial dose of drugs. Molecular tests are available which detect mutation in the chromosomal genes that encode either the catalase gene that mediate resistance to rifampin.

The luciferase assay

Which can detect drug-resistance organisms in a few days. Luciferase is an enzyme isolated from fireflies that produces flashes of light in the presence of ATP. If the organism isolated from the patient is resistance, it will not be damaged by the drug, and the luciferase will produce the normal amount of light. If the organism is sensitive to the drug less ATP will be made and less light produced.

There are two approaches to the diagnosis of latent infections. One is the PPD skin test. Because there are problem both in the interpretation of the PPT test and will the person returning for the skin test to be read quantifiable laboratory based test is valuable. The laboratory test is an interferon gamma assay (IGRA) and there are two version available. This laboratory tests is an interferon-gama almGuerin, the tests not influenced by whether a person has been previously immunized with the BCG vaccine.

TREATMENT and RESISTANCE

Multidrug is used to prevent the emergence of drug resistance mutants during the long 6-9 months duration of treatment organism that become resistant to one drug will be inhibited by the other. These drug INH, Rifampin.
Previous treatments for tuberculosis predispose to the selection of these MDR organisms. Non compliance, the failure of patients to complete the full course of therapy is a major factor in allowing the resistance organisms to survive. One approach to the problem of noncompliance is directly observed therapy (DOT) in which health care workers observe the patients taking the medication.

*Mycobacterium leprae*

The organisms cause leprosy or Hansen's disease.

Important properties

*M. leprae* has not been grown in the laboratory, either on artificial media or in cell culture. It can be grown in experimental animals, such as mice and armadillo appears to be reservoir for human infection in the Mississippi delta region where these animal are common. The optimal T for growth 30°C is lower than body T it is there for grows preferentially in the skin and superficial nerves. It grows very slowly, with a doubling time of 14 days. One consequence of this that antibiotic therapy must be continued for a long time, usually several years.

Laboratory diagnosis

In lepromatous leprosy the bacilli are easily demonstrated by performing an acid-fast stain of skin lesion or nasal scraping. Lipid-laden macrophage called foam cells containing many acid-fast bacilli are resistant to the drug.

In the tuberculoid form, very few organisms do not grow on artificial media. No serologic tests for syphilis, such as the VDRL and RPR test occur frequently in patients with lepromatous leprosy. The diagnoses can be confirmed by using the polymerase chain reaction (PCR).

Treatment

The ministry of therapy is dapsone, but because sufficient resistance to the drug has merged, combination therapy is now recommended e.g. dapsone, rifampin. For the tuberculoid form, treatment is given for at least 2 years or until the lesion are free of organisms.

*Gram positive bacilli and spore Bacillus anthracis*

Disease

Anthrax: three forms:
Medical microbiology Theory
Second year college of pharmacy, all Mustansiriya university, Assistant professor. Dr. Thamer Mutlag

Gastrointestinal

Cutaneous: cut and abrasion in skin

Inhalation anthrax are inhaled

B. anthracis has a tendency to form very long chains of rods and in culture is non motile and nonhemolytic, colonies are characterized by a rough uneven surface with multiple curled extensions at the edges resembling a Medusa head. B. anthracis has a D-glutamic acid polypeptide capsule of a single antigenic type that has antiphagocytic properties. The organism is also a potent producer of one or more exotoxin, which they have been multiple names (lethal factor, edema factor, protective antigen).

Human anthrax is typically an ulcerative sore on an exposed part of the body, the ulcer resolved without complication. If anthrax spores are inhaled, fulminant pneumonia may lead to respiratory failure and death.

Pathogenesis

When spore of B. anthracis reach the rich environment of human tissue they germinate and multiply in the vegetative state. The antiphagocytic properties of the capsule as survival eventually allowing production of large enough amount of the exotoxin to cause disease. Exotoxin have multiple activities.

Diagnosis

Culture of skin lesion, sputum, blood, and CSF are the primary means of anthrax diagnoses. Gram stains of sputum or other biologic fluids showing large numbers of these positive bacilli can indicate the diagnosis. Such acilli are also unusual in sputum. B. anthracis and other Bacillus species are not difficult to grow. In fact clinical laboratories frequently isolate the nonanthrax species as environmental contaminants. The saprophytic species are B. hemolytic and motile these features can be used to exclude B. anthracis. Blood culture are positive in material most cases of pulmonary anthrax. Red Albert genuninetestis a food drug (FDA) immunochromatographic test.

Treatment

Almost all strains of B. anthracis are susceptible to penicillin, which remains the treatment of choice for all forms of anthrax. Doxycycline or ciprofloxacin are alternative and are also recommended for other widespread in the environment, and isolation of one of the more than 20 bacillus species other than B. anthracis from clinical material represent contamination of the specimen. Occasionally B. cereus, B. subtilis, produce genuine infection, including infection of the eye, soft tissues, and lung. Infection is associated with
1-Immunosupresion

2-tauuma.

3-Indewelling catheter

4-Contamination of complex equipment such as n artificll kideny.

B. ceres

Deserve species mention. This species is most likely to cause opportunistic infection, which suggest avirulence intermediate between that of B. anthracis and other species.

Anthrax isolated from abscess has been shown to produce a destructive pyogenic toxin . B.cerus can also cause food poisoning by means of enterotoxins. One enterotoxin acts by stimulating adenyl cyclase production and fluid excretion in the same manners as toxigenic E.coli and Vibrio cholera. Lecithinase production by B. cerus on egg yolk agar by the opaque zone of precipitation around the bacterial Indian inkformation , stain with Indian ink the capsule resistant to staining. The capsule appears as halo the simplebetween

Capsule stain

Grow the organisim on skim milk agar to promote capsule formation, stain withindian ink the capsule resistant to staining. The capsule appears as halo between the simple- stained cellsand the negative-stained background.

Spore staining

End spore in cell appears unstained ovals in the center of stained cells. Acillary, Gram negative rods morphology

Add malachite green,rinse with water and the ad safranin.

Brucella

rods

Species

B. abortus cattle,B melitensis sheep,,goat,B. suis pig, B. canis spread to human from dog

Bactriology
Are small, cocccobacillary are Gram negative rods morphology resemble Haemophylus and Bordetella. They an motile, non acid fast, non spore forming. The cells have atypical G- structure and the outer membrane contains proteins and two major antigenic variants (A,M). Their growth is slow, requiring at least 2-3 days of aerobic incubation in enriched broth or on blood agar. All species produce catalase, oxidase, and urease, but not ferment carbohydrate. They differentiate by carbon dioxide requirements, hydrogen sulphide production, and susceptibility to dyes (thionine and basic fuchsins).

Pathogenesis
All brucella are facultative intracellular parasites of epithelial cells and professional phagocytes. After they penetrate the skin or MM they enter and multiply in macrophage in the liver, sinusoids, spleen, bone marrow.

Diagnosis
Definitive diagnoses requires isolation of brucella from the blood or from biobsy specimens of the liver, bone marrow, L.N. Supplimintation with carbon dioxide is needed for growth of B. abortus. Blood cultures may require 2 to 4 weeks for growth. The diagnoses is made serologically. Antibodies that agglutinate suspension of heat-Killed organismstypically reach titters of 1:640 or more in acute disease. Lower titers m of hpreviousyuman brucellosisay reflect previous disease or cross-reacting antibodies.

Products
Tetracycline, Doxycycline

Prevention
The control of human brucellosis relates directly to prevention programs in domestic animals and avoiding unpasterized milk and milk products. In slaughter houses, important means of prevention include careful wound dressing, protective glasses and clothing, prohibition of raw meat ingestion, and the use of previously infected immune individuals in high risk areas.

P

Adequate

Clostridium

CL. tetani

Disease: Tetanus

CL secrete two toxin

1- tetanospasmin
2-tetanolysin

pore

L.D

There is no microbiologic or serologic diagnosis. Organisms are rarely isolated from the wound site. Clostridium tetani produce a terminal spore at the end of the rode. This gives the organism the characteristic appearance of a tetanus racket G+ or drum stick.

**Treatment**

Maintained and respiratory support.

Tetanus immune globulin (tetanus antitoxin) is used to neutralize the toxin. Adequate airway must be maintained and respiratory support given.

*Clostridium botulinum* blocks release of acetylcholine

**Disease: Botulinum**

**Pathogenesis**

Botulinum toxin types of toxin, type A, B, E are the most

Is absorbed from the gut and carried via the blood periplural nerve synapse where it blocks release of acetylcholine; it is a protease that cleaves the protein involved in acetylcholine release. The toxin is a polypeptide encoded by lysogenic phage. Along with tetanus toxin, it is among the most toxic substances known. There are eight immunologic types of toxin, type A, B, E are the most common in human illness.

L.D

The organism is not cultured. Botulinum toxin is demonstrable in uneaten food and the patient serum by mouse protection tests. Mice are inoculated with sample of the clinical specimen and will die unless protected by antitoxin.

**Treatment**

Trivalent antitoxin (type A, B, E) is given along with respiratory support.

*Clostridium perfringes*

Cause two diseases: gas production/gangrene and food poisoning, depending on the route of entry into the body.
Disease gas gangrene

Myonecrosis, necrotizing fasciitis is one of the two diseases caused by Cl. Perfringes.

Pathogenesis

Organism grow in trauma tissue especially muscle and produce a variety of toxin. The most important is alpha toxin (lecithinase) which damages cell membranes including those of erythrocytes. L.D.

Smears of tiskey tissue and exudates sample show large G+ rods. Spores are not seen because they are formed primarily under nutritionally deficient conditions, the organism are cultured anaerobically and then identified by sugar fermentation reaction and organic acid production. Cl. Perfringes colonies exhibit (double zone of hemolysis on blood agar or egg yolk agar is used to demonstrate the presence of the lecithinase. Serologic tests are not useful.

Treatment

Pencillin G

Clostridium difficile

Disease

Antibiotic – associated pseudomembranous colitis most common nosocomal hospital acquired infection cause of diarrhea.

L.D.

The presence of exotoxin in the filtrate of an patient, stool specimen is the bases of the laboratory diagnosis. It is sufficient to culture the stool for the presence of Cl. Difficile because people can be colonized by the organisms and not have disease. There two tests used to detect exotoxin one is ELIZA, two PCR.

Treatment

Oral metronidazole.

Yersinia pestis

Disease
Plague, black death

It is transmitted to human by bite of the rat flea two form

1- Bubonic

2- Pneumonia

Species Yersinia enterocolitica, Y pseudotuberculosis

Important properties

Y. pestis is a small gram negative rod that exhibit bipolar staining it resemble s a safety pin, with central clear area. Freshly isolated organisms posseses a capsule composed of apolysacharide- protein complex. The capsule can be lost with passage in the laboratory, loss of the capsule is accomplished by a loss of virulence. It is one of the most virulent bacteria known and has a strikingly low ID50 1-10 organisms are capable of causing disease.

L.D

Smear and culture of blood or pus from the bubo is the best diagnostic procedure. Great care must be taken by the physician during aspiration of the pus by laboratory workers doing the culture not to create an aerosol that might transmit the infection. Giemsa or wagson stain reveals the typical safety – pin appearance of the organism better than does Gram stain fluorescent- antibody staining can be used to identify the organism in tissue. Arise in antibody titer to the envelope antigen can be useful retrospectively.

Treatment

The treatment of choice is a combination of streptomycin and tetracycline.

Helicobacter pylori (H. pylori)

Cause Gastritis. H. pylori has morphologic and growth similarities to the campylobacters. The cell are slender, another curved rod, with motil polar flagellae. The cell wall structure is typical of other Gram negative bacteria, although H. pylori LPS may be less toxic than its enteric counterparts. Growth requires microaerophilic atmosphere and is slow 3 to 5 days urease positive whose action allows the organisms to persist in low the generation, of ammonia. A nther secreted protein called the vacuolating
Cytotoxin (VACA) causes apoptosis in eukaryotic cells; it enters generating multiple large cytoplasmic vacuoles.

**Pathogenesis**

Multiple mechanisms to adhere to the gastric mucosa and survive the acid milieu of the stomach. Motility provided by the flagella allows the organisms to swim to the less acid pH locale beneath the gastric mucosa, where the urease forms a neutral microenvironment by ammonia production. At the mucosal adherence is mediated by surface protein one of which binds to Lewis blood group antigen, present on the surface of gastric epithelial cells. Prolonged and aggressive inflammatory response could lead to epithelial cell death and ulcer.

**Diagnosis**

The most sensitive means of diagnosis, with biopsies and culture of the gastric mucosa. The H. pylori urease is so potent its activity can be directly demonstrated in biopsies in less than an hour. Non-invasive methods include serology and urea breath test. For the breath test, patients ingest C13 or C14-labeled urea, from which the urease in the stomach produces products that appear labeled O2 in the breath. A number of methods for detection of antibody directed against H. pylori are not available. Because IgG or IgA remain elevated as long as the infection persists.

**Treatment**

Urease test

Preemptive diagnosis at the time of endoscopy is the biopsy urease test, in which ground biopsy material is added to Christensen's urea broth, providing a strong shift in pH to alkalinity and rapid color change if large numbers of H. pylori are present.

**Campylobacter**

**Disease**

Food poisoning, gastroenteritis.
C. jejuni is a frequent cause of enteritis, especially in children. C. jejuni infection is common antecedent to Guillain-Barré syndrome (GBS). Other campylobacter species are rare causes of systemic infection particularly bacteremia.

**L.D**

If the patient has diarrhea, a stool specimen is cultured on a blood agar plate containing antibiotics that, 5% oxygen and 10% carbon dioxide, which favors the growth of C. jejuni. It is identified by failure to grow at 25°C, oxidase positive and sensitivity to nalidixic acid. Unlike shigella and salmonella, lactose fermentation is not used as a distinguishing feature. If the bacteremia is suspected, a blood culture incubated under standard temperature and atmosphere conditions will reveal the growth of the characteristically comma – or S. shaped, motile, Gram negative rods. Identification of the organisms as C. intestinalis is confirmed by its failure to grow at 42°C. Its ability to grow at 25°C and its resistance to nalidixic acid.

**Treatment**

Erythromycin or ciprofloxacin is used successfully in C. jejuni enterocolitis. The treatment of choice for C. intestinalis bacteremia is an aminoglycoside.

**Prevention**

There is no vaccine or other specific preventive measures proper sewage disposal and personal hygiene (hand washing are important.

**GBS**

Complication of infection a disorder affecting the peripheral nervous system, ascending paralysis, lidum

**Spirochetes**

**Treponema pallidum**

**Disease**

**Syphilis**

T. pallidum has not been grown or bacteriologic media or in cell culture. Non pathogenic treponema, which are part of the NF of human MM, can be cultured. T. pallidum grow very slowly. The medical important of that fact is that antibiotics must be present at an effective level for several weeks to kill the organism and cure the disease. The antigen of T. pallidum induce specific antibodies which can be detected by immunofluoresence or haemaglutinin tests in the clinical laboratory. They also induce nonspecific antibodies (regin), which can be detected by the flocculation of lipid cardiolipin extracted from normal mammalian tissue e.g beef heart.
Pathogenesis

1- primary syphilis local chancre

2- Secondary syphilis rash on palm and soles or genital are called condylomata.

3- latent
   a- early
   b- late

4- Tertiary show granulomas gummas especially of skin and bones. CNS involvement or cardiovascular lesions

5- Congenital: The organism across the placenta typically after the third month of pregnancy and fetal infection can occur. In the infected neonates, skin and bone lesions, hepatosplenomegaly, interstitial keratitis.

L.D

There three important approaches

1- Microscopy

Spirochetes are demonstrated in the lesion of primary or secondary syphilis, such as chancre or condylomata, by darkfield microscopy or by direct fluorescent antibody (DFA) test. They are not seen on Gram stained smear. In biopsy specimens, such as those obtained from the gum mass seen in tertiary syphilis, histologic stain such as silver stain or fluorescent antibody can be used.

2- Non specific serologic test

These tests involve the use on non treponemal antigens.

Extract of normal mammalian tissue e.g. cardiolipin from beef heart react with antibodies in serum sample from patients with syphilis. Flocculation of these antibodies. The titer of these nonspecific antibodies decreases with effective use of treponemal antigen treatment tests.

3- Specific serologic tests

These tests involve the use of treponemal antigens and therefore are more specific than those above.

In these tests T. pallidum reacts in immunofluorescence (FTA, ABS or haemagglutinin assay with specific treponemal antibodies in the patients serum.)
Treatment

Penicillin is effective in the treatment of all stages of syphilis.

Borrelia burgdorferi

Disease: Lyme disease

By the bite of tick

Important properties

Is aflxiable, motile spirochetes that can be visualized by darkfield microscopy and by Giemsas stain and silver stains. Culture of the organisms from the tick vector is positive.

B. recurrentis cause relapsing fever

L.D

The diagnosis is typically made serologically by detecting either IgM antibody or rising titer of IgG antibody with ELISA, PCR that detect the organism DNA is also

Treatment

Amoxicillin

Leptospira interrogans

Are coiled, fine spirochetes that are non stained with dyes but are seen by dark field microscopy.

Diagnosis

1- is based on history of possible exposure, suggestive clinical signs

2- Marked rise an agglutination antibody titers.

3- Occasionally are isolated from blood and urine culture.

Human infection results when leptospiaras are ingested or pass through MM or skin. They circulate in the blood and multiply in various organs, producing fever and dysfunction of the liver jaundice, kidney uremia haemorrhage CNS meningitis.

Treatment penicillin G

Rickettsia
The Rickettsia are a group of organisms that infect wild animals, with humans acting as accidental hosts in most cases. Most of these organisms are passed between animals by an insect vector. All Rickettsia serologically.

Symptoms
Fever, headache, and rash.

All rickettsiae are pleomorphic Gram-negative coccobacilli. The organisms multiply by binary fission in the cytoplasm of host cells, which are finally lysed during the release of rickettsia.

Early diagnosis is made on clinical grounds based on the symptoms of fever, rash, and exposure to ticks. The characteristic spread of rash from the extremities to the trunk helps distinguish Rocky Mountain spotted fever (RMSF) from meningococccemia.

L.D.

Biopsy specimens of skin tissue from the rash of RMSF can be stained directly with an indium immunofluorescence reagent. The Giemenez stain is also used for examining clinical material. Although the rickettsia can be cultured in embryonated eggs and in tissue culture, diagnosis is primarily accomplished serologically.

The test is Weil Felix reaction, the fortuitous agglutination of certain strains of Proteus vulgaris by serum from patients of which M with Rickettsial disease.

Coxiella burnetii
Disease Q fever
Coxiella is passively phagocytized by host cells and multiplies with vacuoles.

Coxiella is inhaled into the alveoli picked up by macrophages, and carried to lymph nodes from which it disseminates into the bloodstream, granulomatous hepatitis, and endocarditis are common sequelae.

C. burnetii undergoes an antigenic phase variation during infection. The organism exits in phase 1 during initial infection, and humans produce antibody to phase 1 early in disease, such as endocarditis or hepatitis; antibodies to phase 1 are present and can be measured by complement fixation or immunofluorescence.

Mycoplasmas

Mycoplasma are a group of small, wall-less organisms, of which M. pneumonia is the major pathogen. M. pneumonia on artificialia caused typical pneumonia.
important properties

Are smallest free living organisms. Their most striking feature is the absence of a cell wall. M stain poorly with Grams stain, and antibiotics that inhibit cell wall synthesis, e.g. penicillin and cephalosporin, are infective. There is the only bacterial M contains cholesterol, a sterol found in eukaryotic cell M. M can be grown in the laboratory on artificial media, but they have complex nutritional requirements including several lipid.

They grown slowly and require at least 1 week to form visible colony. The colony has characteristic fried egg shape, with a raised center and their outer edges.

L.D

Diagnoses is not made by culturing sputum sample. It takes at least 1 week for colonies to appear on special media. Serologic testing is the mainstay of diagnoses. A cold agglutination of 1:128 or higher is indicative of recent infection.

Treatment

Erythromycin

Chlamydia

Species: C. psittaci, C. pneumonia, C. trachomatis pneumonia

Like, elementary bodyspores

Disease

Chlamydia begins when psittacosis cause P. sittacosis, C. trachomatis causes eye respiratory and genital tract infections. C. trachomatis is the most common cause of sexually transmitted disease pneumonia. Called TWAR (cause atypical pneumonia)

Important properties

Chlamydia are obligate intracellular bacteria, C have a replication cycle such as different from that of all other bacteria. The cycle begins when the extracellular metabolically inert, spore like elementary body enters the cells and recognizes into a large metabolically active reticulate body. The latter and does repeated binary fission to form daughter elementary bodies. Which are released from the cell within the cells, the site of replication appears as an inclusion body, which can be stained and visualized microscopically. These inclusions are useful in the diagnosis of these organisms in the clinical specimens laboratory.

L.D

C form cytoplasmic inclusion, which can be seen with special stain Giemsa stain or by immunofluorescence. The gram stain is visualized not useful. In exudates the organisms can be identified with epithelial cells. By fluorescent antibody staining or hybridization with a DNA probe. Can be
grow in cell cultures treated with cycloheximide which inhibit host cell but not chlamedia protein synthesis. In culture C. trachomitis forms inclusions containing glycogen whereas C psittaci and C pneumonia form inclusion that don’t contain glycogen. The glycogen filled inclusions are visualized by staining with iodine.

Treatment
All are susceptible to tetracycline and erythromycin.

Pasterella multocida

Are part of animal flora and are transmitted to humans during close animal contacts including bites, virulence factors are not recognized and the organisms may be considered opportunistic pathogens that requires mechanical destruction of the host anatomic barriers, such as occurs with bite induced wounds.

Direct detection method
Pasterella species are typically short, straight bacilli. The bacteria grow on 5% sheep blood and chocolate agars. Most strains do not grow on MacConkey agar and incubated at 37°C in carbon dioxide or ambient air for a minimum of 24 hours. Hemolysis and odor on blood agar. The Pasterella should be oxidase positive, based on the use of tetra methyl-phelene diamine dihydrochloride reagent.

Serodiagnosis technique not used for the laboratory diagnoses of infectious caused by the organisms.

Prevention
Because these organisms doesn’t a threat to human health, there are no recommended vaccination or prophylaxis protocols.

Francisella
Disease Tularemia
incubation this requirement for a complex medium for isolation and growth. The organism are faintly staining, gram negative coccobacilli that are non motile and obligate aerobic. F. tularensis is carried by many species of wild rodents, rabbit. Human become infected by handling the carcass on skin of infected animals, through insect vectors. The capsule appears to be necessary component for expression of full virulence, allowing the organism to avoid immediate destruction by polymorphonuclear neutrophils.

Diagnoses
Microscopy is intensive
Culture on cysteine-supplemented media, chocolate agar, is sensitive if prolonged incubation is used and specific. Serology can be used to confirm the clinical diagnosis.

Treatment
Gentamicin