Medical microbiology Theory
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Mycobacterium tuberculosis(T.B)Medical microbiology theory, second year ,DR thamer

L.D

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

F or rapid screening purposes, auramine stainen flouresence microscopy is used after digestion of the specimens by treatments with NaoH and concentration by centrifugation, the naturalis cultured on special media. Such as Lowenstein-jensen agar, for up 8 weeks. It will not grow on ablood agar plate. In liquid medium is preferred for isolation because the organisims grows more rapidly and reliably then it does on agar. If growth in the culture occurs, the organisim can be identified

M.tuberculosis produce niacin, wherese almost no other mycobacteria do. It also produce catalase. Nucleic acid amplification test can be used to detect the presence of M. tuberculosis directly toin clinical specimens are highly specific , such as sputum. Tests are available that detect either the ribosomal RNA or the DNA of the organisim. These test are highly specific, but their sensitivity varies.Because during resistance especially to isoniazid is aproplem,susceptibility tests should be performed. The organisims 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.

Theluciferase assay

Which can detect drug- resistance organisims in afew days. Luciferase is an enzyme isolated from fireflies that produces flashes of light in the presence of ATP. If the organisim 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 organisim 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 returing for the skin test to be read aquantifiable laboratory based test is valuable. The laboratory test is an interferon gamma assay (IGRA) and there are two vesion available . This laboratory tests is an interferon-gama almGuerin, the tests not influenced by whether aperson 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 organisim 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 organisims. Non compliance . the failure of patients to complete the full coarse of therapy is amajor factor in allowing the resistance organisims to survive. One approach to the proplem of noncompliance is directly observed

therapy (DOT) in which health care workers observe the patients taking the medication .

Mycobacterium leprae

The organdm cause leprosy or hansens disease a

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 armandillo appears to be reservoir for human infection in the Missippi 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 adubling time of 14 days. One consequence of this that antibiotic therapy must be continued foralong time,usually several years.

Laboratory diagnosis

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

In the tuberculoid form , very few organisim does not geow on artificial media. No serologic tests for syphilis , such as the VDRL and RPR test occur frequently in patients with lepromatus 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, cominaion therapy is now recommended e.g dapsone,rifampin. For the tuberculoid form. Treatment is given for at least 2years or until the lesion are free of organisim,

<u>Gram positive bcilli e spore Bacillus anthracis</u>

<u>Disease</u>

<u>Anthrax:three form:</u>

Gastrointestinal

Cutaneous:cut and abrasion in skin

Inhalation anthrax are inhaled

B.anthracis has aatendensy to form avery long chains of rods and in culture is non motile and nonhemolytic,colonies are characterized by arough uneven surface with multiple curled extensions at the edges resembling aMedusa head . B.anthracis has aD-glutamic acid polypeptide capsule of asingle antigenic type that has antiphagocytic properties. Theorganisim is also apotent 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 expoasedpart 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 environmen of huan tissue they germinate and multiply in the vegetative state. Theantiphagocytic properties of the capsule as insurvivalenventually allowing productionof 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. Infact cnlinical laboratories frequently isolate the nonanthrax species as environmental contaminants. The saproephytic speciesare 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 afood drug(FDA) immunochromatog raphic test.

Treatment

Almost all strains of B.anthracis are susceptible to pencillin, which rema, softins the treatment of choice for all forms of anthrax . Doxycycline or ciprofloxacin are alternative and ar B.e also recommended for

Other arewide spread in the environment, and isolation of one of the more than 20 bacillus species other thanB.anthracis from clinicalmaterial representcontamination of the specimen. Occasionally B,cereus, B.subtilis,produce genunine infection,including infectionof the eye, soft tissues,and lung. Infection is associatedwith

1-Immunosupresion

2-tauma.

3-Indewelling catheter

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

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 adestructive 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 opague 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, coccobacillary are Gram negative rods morphology resemble Haemophylus and Bordetella. They anon motile , non acid fast, non spore forming. The cells have atypical G- structure and the outer membrane contains proteins and two major antigenic varients (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 fachsin)

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 marrowor L.N. Supplimintation with carbon dioxide is needed for growth of B.abortus . Blood cultures may require2 to 4 weeks for growth. The diagnoses is made serologically. Antibodies that agglutinate suspention of heat- Killed organisimstypically reach titers of 1:640 or morein acute disease. Lower titers m of hpreviouslyuman 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 avoidingunpasterized 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 ofpreviously infected immune individuals in high risk areas

p

. Adequte

Clostridium

CL.tetani

Disease:Tetanus

CL secreate two toxin

1-tetanospasmin

2-tetanolysin

pore

L.D

There is no microbiologic or serologic diagnosis. Organisims are rarely isolated from the wound site. CL.tetani produce aterminal spore aspore at the end of the rode. This gives the organisims the characteristic appearance G of atetanus racket G+ or drum stick.

Treatment

maintained and respiratory supportnt.

Tetanus immune globulin(tetanus antitoxin) is used to neutralize the toxin. Adequate airway must be

Maintained and respiratory supportgiven.

Clostridium botuit blocksnumes release acetylecholine

Disease:Botulinum

Pathogenesis

Botulinum toxintypes of toxine, typeA,B,E are the most

Is absorbed from the gut and carried via the blood periplural nerve synapse whereit blockes release of acetylecholine,it is aprotease that cleaves the protein involved in acetylecholine release. The toxinis polypeptide encoded by lysogenic phage. Along with tetanus toxin ,it is among the most toxic substance knortwn. There are eight immunologic types of toxin, type,A,B,E are the most ommon in human illness.

L.D

The organisims 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(typeA,B,E) is given along with respiratory support.

Clostridium perfringes

Cause two disease:gass productiongangrene and food poisoning, depending on the route of entery into the body.

Disease gass gangrene

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

Pathogenesis

Organisim grow in trauma tissue especially muscle and produce avariety of toxin. The most important is alpha toxin(lecithinase) which damage cell membranes including those of ily

L.D

Smears of tilyrie tissue and exudates sample show large G+ rods. Spores are not seen because they are formed primarily under nutritionally deficient conditions, the organisim 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 lecithinace. 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 filterate of apatient, stool specimen is the bases of the laboratory diagnosis . It is sufficient to culture the stool for the presence of CL. Difficile because people ca be colonized by the organisims and not have disease. There two tests used to detect exotoxin one is ELIZA,two PCR.

Treatment

Oral metronidazle.

Yersinia pestis

Disease

Plague,black death

It is transmitted to human by bite of the ratflea two form

1-Bubonic

2-Pneumonia

Species Yersinia enterocolitica,Y pseudotuberculosis

Important properties

Y. pestis is asmall gram negative rod that exhibit bipolar staining itresemble s asafty pin, with central clear area. Freashly isolated organisims posseses acapsule composed of apolysacharide- protein complex. The capsule can be lost with passage in the laboratory, loss of the capsule is accomplished by aloss of virulence. It is one of the most virulent bacteria kown and has astrinkling low ID50 1-10 organisims 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 phsian during aspiration of the pus by laboratory worken dowing the culture not to create an aerosol that might transmit the infection. Giemsa or wagson stain reveals the typical safty – pin appearance of the organisim better than does Gram stain flouresent- antibody staining can be used to identify the organisim in tissue. Arise in antibody titer to the envelop antigen can be useful retrospectively.

Treatment

The treatment of choice is acombination of streptomycin and tetracycline.

Helicobacter pylo ri(H.pylori)

Cause Gastritis.H.pylori has morphologic and growth similarities to the campylobacters. The cell areselender,cnotherurved 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 amicroaerophilic atmosphere and is slow 3to 5 days urease positive whose action allows the organisims to persist in lowy the generation,of ammonia. A nother secreted protein called the vacuolating

cytotoxin(VACA) causes apotsis ineukaryotic cells it enters generating multiple large cytoplasmic vacuoles.

Pathogenesis

Multiple mechanisims to adhere to the gastricmucosa aresponse a nd survive the acid milieu of the stomash.Motility provided by the flagella allows the organisims to swim to the less acid pH locale beneth the gastric mucosa, where the urease amore neutral microenvironment byamonia production. At the mucosaadherence is mediated by surfaceprotein one of which binds to lewis blood g roup antigen, present on the surface of gastric epithelial cells. Aprolonged and aggresve inflammatoryresponse could lead to epithelial cell death and ulcer.

Diagnosis

The most sensitive means of diagnosis, with biobsy and culture of the gastric mucosa. The H.pylori

Urease is so potent its activity can be directly demonstrated in biopsies in less than anhour. No

Invasive methods include serology and aurea breath test . For breath test, the patients ingest C13or C14 – labeled urea, from which the urease in the stomach produce products that appears labeled o2 in the breath. Anumber of methods for detection of antibody directed against H.pylori are non available. Because igGor igA remain elevated as long as the infection persists.

Treatmentesem

Urease test

Presemptive diagnosis at the time of endoscopy is the biobsy urease test, in whichground biopsy material is added to christensens urea broth,providing astrong shift in ph to alkalinity and rapid color change if large number of H. pylori are present.

Campylobacter

DISEASE

Food poisoning,gastroenteritis.

C.jejuni is afrequient cause of enteritis , especially in children. C.jejuni infection is common antecedent to Guillain-Barrs syndrome(GBS). Other campylobacter species are rare causes of systemic infection particularly bacterimia.

L.D

If the patient has diarrhea, astool specimen is cultured on ablood 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 25c, oxidase positive and sensitivity to nalidixic acid. Un like shigella and salmonella, lactose fermentation is not used adistigushing features. If the bacterimia is suspected, ablood culture incubated under standard temperature and atmosphere conditions will reveal the growth of the characteristically comma – or S. shaped , motile, Gram negative rodes. Identification of the organisims as C. intestinalis is confirmed by its failure 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 adisorder affecting the peripheral nervous system,ascending paralysis, lidum

Spirochetes

Treponema palidum

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 organisim and cure the disease. The antigen of T. pallidum induce specific antibodies which can be detected by immunoflouresence or haemagglutinint 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

!-primary syphilis local chancre

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

3-latent

a-early

b-late

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

5-Congenital : The organisim across the placenta typically after the third month of pregnancy and fetal infection can occur. In the infected neonates, skin and bone lesions,hetreatmentpatosplenomegaly,interstitiaalkeratitis.

L.D

There three important approaches

1-Microscopy

Spirochetes are demonstrated in the lesion of primary or secondary syphilis, such as chancres orcondylomata , by darkfield microscopy or by direct flouresent antibody(DFA) test. They are not seenonG staned smear. In biopsy specimens, such as those obtained from the gum mass seen in tertiary syphilis, histologic stain such as silver stain or flouresent antibody can be used.

2- Non specific serologic test

These tests involve the use on nonterponemal antigens.

Extract of normalmammalian tissue e.g cardiolipin from beef heart react with antibodies in serum sample from patients with syphilis. Flocculatof these antibodies. The titer of these nonspecific antibodies decreases with effecti the use of treponemal antigenve 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 immunoflourescence (FTA, ABS or haemagglutinin assay with specific treponemal antibodies in the patients serum .

Treatment

Pencillin 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 microscopyand by Giemsas stain and silver stains. Culture of the organisims from the tick vector is positive.

B.recurrentis cause relapsing fever

L.D

The diagnosis is tepically made serologically by detecting either igM antibody or arising titerof igG antibody with ELISA, PCR that detect the organisim 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 leptospiras 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 pencillin G

Rickettsia

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

Symptoms

Fever,headach,and rash.

All rickettsia are pleomorphic Gram negative coccobacilli. The organisims multiply by binary fisson in the cytoplasm of host cell, which are finally lysed during release rickettsia.

Early diagnosis is made on clinical ground based on the symptoms of fever, rash and exposure to ticks. The characteristic spread rash from the extrimities to the trunk help to distinguish Rocky mountain spotted fever (RMSF).from meningococcemia.

L.D.

Biopsy specimens of skin tissue from the rash of RMSF can be stained directly with aspecific edimmunoflourescence reagent. The Giemenez stain is also used for examination of clinical material. byAlthough the rickettsia can be cultured in embryonated egg and in tissue culture. Diagnosis is primarly accomplished serologically.

The test is Weil felix reaction ,the fortuitions 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 cell and multiplies with vacuoles.

Coxiella is inhaled into the alveoli picked up by macrophage, and carried to lymph node frm which it dissiminated into the blood stream granulamatus hepatitis and endocarditis are common squeal.

C. burnetti undergoes an antigenic phase variation during infection. The organisim exits in phse 11 during initial infection, and humans prduce antibody to phace 11 early in disease, such as endocarditis or hepatitis, antibodies to phase 1 are present and can be measured by complement fixation or immunoflourescence .

Mycoplsmas

Mycoplasma are group of small, wall less organisims, of which M.pneumonia is the major pathogens .M.pneumon on artificialia caused typical pneumonia

important properties

Are smallestfree living organisim. Their most striking features is the absence of acell wall . M stain poorly with Grams stain, and antibiotic that inhibit cell wall synthesis, e.g pencillin and cephalosporin, are infective. There is the only bacterial M contains cholesterol, asterol found in eukaryotics cell m. M can be grown in the laboratory on artificialmedia, but they have complex utritional requirements including several lipid.

They grown slowly and reqire at least 1week to frm visible colony. The colony has characteristic fried egg shape, with araised center and atheir outer egges.

L.D

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

Treatment

Erythromycin

Chlamydia

Species:C.psittaci,C. pneumonia, C. trachomatispneumonia

Like, elementary bodyspore

Disease

Chlamydia begins whenpsittacosis cause P.sittacosis,C. trachomatitis causes eye respiratory and genital tract infections. C .trachomatitis is the most common cause of sexually transmited disease.pneumonia called TWARcause atypical pneumonia
Important properties
Chlamydia are obligate intracellular bacteria, C have areplication cycle such as different from that of all other bacteria. The cycle begin when the extracellular metabolically inert, spore like elementary body enters the cells and recognizesinto alarge metabolicallyactive reticulate body. The latter andeoes repeated binary fission to form daughter elementary bodies. Which are released from the cell within the cells, the site of replication appears is an inclusion body, which can be stained and visualized microscopically. These inclusions are usefull in the diagnose of these oranisims in the clinical specimens laboratory.
L.D
C form cytoplasmic inclusion, which can be seen with special stain Giemsas stain or by immunoflourescence. The gram stain ivisulizeeeddd s notusefull. In exudates the organisims can be identified with epithelial cell. By flouresent antibody staining or hybridization with aDNA 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 organisims may be considered opportunistic pathogens that requires mechanical distruction 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 strain do not grow on MaCconkey agar and incubated at 37 c in carbon dioxide or ambint air for aminimum 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 organisims.

Prevention
Because these organisims doesn't athread to human health, there are no recommended vaccination or prophylaxis protocols.

Francisella
Disease Tularemia
incubationthis requirement for acomplex medium for isolation and growth. The organisim are faintly staining, gram negative coccobacilli th. at are non motile and obligate aerobic. F.tulrensis is carried by many species of wild rodents, rabbit. Human become infected by handling the carcass on skin of infected animals, through insedt vectors. The capsule appears tobe necessary component for expression of full virulence, allowing the orgnisim to avoid immediate destruction by polymorphoneuclear neutrophils.
Diagnoses
Microscopy is intensive
Culturrreon cysteine- supplemented media,chocolate agar, is sensitive if prolonged incubation is usedand specific. Serology can be used to confirm the clinical diagnosis.

Treatment

Gentamicin