

Gram Positive Rods

Corynebacterium Bacillus Listeria Erysipelothrix



Corynebacterium

- Over 20 species, most are saprophytic
 - Human normal flora, especially skin and nares
- Most grow well on 5% Sheep's blood agar
- Gamma hemolytic gray colonies
- Catalase enzyme positive
- "Diphtheroid" morphology, Gram positive rods in Chinese letter forms
- No spores produced







Corynebacterium diphtheriae

- Agent of Diphtheria
 - Forms tough oropharyngeal membrane (Diphtheroid is Greek for leather), bleeds when attempt to remove
 - Phage mediated exotoxin is distributed from the membrane causing respiratory paralysis, heart, nervous system, and kidney damage
- Non-toxin producing strains cutaneous ulcers and lesions
- Diagnosis: culture, toxin detection (Elek plate), serology, and PCR to study toxin genes
- Grows well on 5% Sheep's blood agar, gray gamma hemolytic colonies, catalase enzyme positive
- Selective medium Cysteine Tellurite agar / black colonies
- Metachromatic storage granules when grown on Loeffler's media and stained with methylene blue
- Treatment: Anti-toxin and Erythromycin or Penicillin



Diphtheria - notice the pseudomembrane in the posterior pharynx. It can become very large and may obstruct the airway.



Corynebacterium diphteriae on Tinsdale agar: black colonies with brown halo





Other Corynebacterium spp.

- Corynebacterium jeikeium
 - Normal human skin flora / thrives on lipid
 - Infects patients with indwelling plastic catheters and devices and can lead to bacteremia
 - Biofilms are formed on the plastic, protecting the organisms from antibiotic treatment
 - Susceptible to vancomycin and tetracycline
- Corynebacterium urealyticum
 - Urease positive
 - Cause of urinary tract infection in post renal transplants
 - Resistant to many antibiotics but vancomycin susceptible







Red is (+) for

Urease reaction

Bacillus species

- Large Gram positive rods with square ends
- Can be easily over-decolorized and appear reddish on Gram stain
- Spores produced which cause clearing in bacillus on Gram stain
- Catalase enzyme positive



Spores/ clear areas in bacillus



Variable staining / not clearly Gram positive



Bacillus anthracis

- Agent of anthrax in herbivores
- Category A agent priority pathogen, potential agent of bioterrorism
- Virulence factors: anthrax toxin and capsular polypeptide
- Infections:
 - Wool sorter's disease acquired from handling contaminated hides, produces a unique black eschar skin lesion
 - Systemic infections: Pneumonia, sepsis, and meningitis
- Irregular shape to colony border on 5% Sheep's blood agar (BAP)
 - Medusa head colonies Gamma (no) hemolysis on BAP Non-motile
 - Susceptible to penicillin











Bacillus cereus

- Most common species causing infection
- Saprophytic environmental organism



- Nosocomial and opportunistic infections, particularly in immunocompromised patients, and patients with indwelling or implanted devices
 - Can be a contaminate in blood cultures
 - Skin infections in Intravenous drug users
- Associated with food poisoning due to emetic toxin production and diarrhea from diarrheal toxin
- Gram positive rod with spores
 - Dull gray colony producing beta hemolysis on BAP
 - Catalase positive
 - Motile
 - Resistant to penicillin





Listeria monocytogenes

- Small Gram positive rod / no spores produced
- Catalase enzyme positive
- Grows well on BAP producing subtle beta hemolysis
- Motility provides identifying information
 - More motile at 25°C than 35°C
 - Tumbling motility on wet mount examination
 - Umbrella motility when inoculated into tubed media
- Bacteremia and meningitis in immune suppressed, pregnancy, neonates, and elderly
 - Culture or molecular amplification methods for diagnosis
- Grows well at 4°C / acquire infection from refrigerated foods
 - Non pasteurized cheese and milk products, Deli case foods
- Ampicillin drug of choice
 - Intrinsic resistance to Cephalosporin antibiotics







Erysipelothrix rhusiopathiae





H2S production detected on Triple sugar iron agar (TSI)

- Small pleomorphic Gram positive rod
- No spores produced
- Catalase enzyme negative
- Alpha hemolytic colony on 5% sheep's blood agar plate
- Only Gram positive rod that produces hydrogen sulfide (H2S) MALDI-TOF is necessary for this difficult to identify bacteria
 - Infections from nature and animals, mostly swine, so is an occupational disease of farmers and butchers
 - Virulence factor: capsule
 - Soft tissue infection and bacteremia +/- endocarditis
 - Intrinsic resistance to Vancomycin





Erysipelas – lacy skin lesions of swine



Gram negative bacilli



• Escherichia coli

- Normal flora in human intestine
- #1 cause of UTI [@80% of cases]
- Bacteremia, neonatal meningitis, and abdominal infections





Indole positive

- MacConkey agar / Lactose fermenter
- Spot indole reaction = positive / turns robin's egg blue
 - Detects breakdown of tryptophan from growth on BAP
- Eosin methylene blue agar (ÉMB) green sheen produced
- Pathogen of diarrhea:
 - Enterotoxigenic (ETEC) E. coli cause of traveler's diarrhea
 - Enterohemorrhagic E. coli (EHEC) (such as 0157:H7)
 - Bloody diarrhea acquired from eating undercooked beef
 - Pathogenicity from Shiga toxin production
 - Hemolytic uremic syndrome (HUS) can result with hemolytic anemia, thrombocytopenia, and renal failure] particularly in young children
 - Culture on MacConkey agar with sorbitol (not lactose) / does NOT ferment sorbitol/ most all E. coli except EHEC ferment sorbitol





Enteric GNRs that ferment Lactose

Enterobacter species

- Enterobacter cloacae complex, most common species
- Environmental GNRs with low pathogenicity
- Usually infects a compromised host
- Enterobacter (Cronobacter) sakazakii associated with neonatal meningitis

Klebsiella species

- *K. pneumoniae* most common species
- Mucoid colony due to capsule production
- Currant jelly sputum produced in alcoholics due to blood mixed with Klebsiella capsular polysaccharide in sputum











Enteric GNRs that do NOT Ferment Lactose!

• Proteus species

Colonies swarm in layers on agar surface

- *Proteus vulgaris* spot indole positive
- Proteus mirabilis spot indole negative
- Normal flora in intestine
- Common cause of UTI and abdominal infections

Serratia marcescens

- Produces red pigmentation which can intensify at room temp
- Environmental contaminate
- Causes infection most usually in
 - Immune suppressed
 - Ventilator associated pneumonia
 - Bacteremia









Triple Sugar Iron Agar (TSI)– Used to detect fermentation of glucose, lactose and/or sucrose and production of hydrogen sulfide [H2S] in GNRs

CHO Fermentation= yellow medium **Gas production**= Disruption of the agar



No CHO fermentation = Red medium

1. Glu/lac/suc fermented with gas

2. Glucose 3. Glucose fermented fermented with H2S

e 4. No CHO d fermentation Non fermenter

• Salmonella species

- Diarrhea with +/- fever and PMNs in the stool
- Infection from eating contaminated food (raw eggs, poultry, ground beef or dairy) or direct contact with a sick animal
 - Must ingest large #'s of organisms to make you ill (1,000,000 bacteria), normal levels of stomach acid is protective
- MacConkey agar does not ferment lactose
- Produces hydrogen sulfide on selective agars
- Motile
- Identification based on biochemical reactions and serologic typing
 - Kaufman White serologic typing for speciation of Salmonella
 - O Somatic (cell wall) antigen Salmonella group "B"
 - H flagellar antigens 2 phases [h1 & h2]
 - Vi capsular antigen Salmonella typhi only











Salmonella typhi

- Typhoid fever high fever and sepsis, no diarrhea
- Human pathogen most cases in US (75%) from international travel
- Post typhoid fever there can be carriage in gallbladder with passage in feces. Can transmit by bad hygiene and food preparation
 - Ingested organisms enter the bowel, move into the blood stream and eventually the bone marrow
 - Diagnosis: Blood cultures and in late stages (>1 month) bone marrow culture
- Vi capsular antigen unique to S. typhi
- Moustache of Hydrogen sulfide (H2S) produced in TSI medium slant



Shigella

- Diarrhea, +/-vomiting, fluid loss, PMNs and blood in stool
- Infection: Human to human transmission /control with good hand hygiene
- Ingestion of low #'s of organisms make you ill [10 100 bacteria]
- No lactose fermentation on agar
- Non motile
- No Hydrogen sulfide (H2S) produced
- 4 species based on somatic (cell wall) antigen
 - S. boydii
 - S. dysenteriae
 - S. flexneri
 - S. sonnei

Group C Group A Group B Group D







Salmonella Shigella Agar (SS agar)

Shigella are colorless due to lactose not being fermented. **Salmonella** does not ferment lactose, but H2S produced by Salmonella turning the colony black



Hektoen agar – Salmonella produces H2S [Hydrogen sulfide] producing black colonies Shigella – green colonies Normal flora – orange colored due to fermentation of lactose (E. coli)



Yersinia enterocolitica

- Major reservoir swine
- Humans infected by eating raw or undercooked pork
- Infections:
 - Diarrhea
 - Septicemia in patients with iron overload syndromes
 - Mesenteric adenitis Infection symptomatic for right lower abdominal pain which mimics appendicitis
 - Infected blood products from transfusion have been reported
- Oxidase = neg, Indole = neg, Urease = pos, Grows well at 4 °C
- CIN agar (Cefsulodin-irgasan-novobiocin) selective agar for Y. enterocolitica





Yersinia pestis

- Plague, Category A agent call public health!
- Obligate flea/ rodent/ flea cycle in nature



- Human infection usually from a rat flea bite leads to Bubonic plague that is infection of the lymphatic system, forms painful buboes (lymph node swelling) at site of the bite
- Hemorrhagic lymph nodes spread Y. pestis into blood stream
- Pneumonia develops from blood stream infection
- Fatality >=50%
- Endemic in SW USA
- Grows well on blood agar
 - catalase +, oxidase -
 - Bipolar staining "safety pin"





• Vibrio cholera

- Natural environment is saltwater
 - Halophilic (salt loving) salt enhances growth
- Rice water diarrheal stool from mucus flecks (classic)
- Virulence due to enterotoxin production
 - Receptor on epithelial cell in small bowel -
 - Activates adenyl cyclase which
 - Increases cAMP with hyper secretion of NaCl and H20
 - Death from dehydration and metabolic acidosis
- Curved "C" shape Gram negative rod
- Selective medium thio citrate bile sucrose agar, (TCBS) yellow color from sucrose fermentation
- Oxidase = positive , grows in 1% salt solution



ig. 20.18 Rice water stool in cholera. (Courtesy of AM Geddes.)



TCBS Agar





- Vibrio parahaemolyticus
 - Diarrhea from ingestion of raw oysters
 - Usually self limited but serious in immune suppressed
 - TCBS medium sucrose fermentation negative so green colony
- Vibrio vulnificus
 - Infection from ingestion of raw shellfish
 - Leads to:
 - Diarrhea
 - Skin infection from injury in water
 - Bacteremia
 - Formation of painful skin lesions on lower extremities with muscle necrosis
 - In patients with liver disease (cirrhosis) and patients with increased serum iron, 50% fatality rate





Acinetobacter baumannii

- Environmental saprophyte and normal flora on human skin
- Gram negative coccoid-bacilli
- Does not ferment lactose / Oxidase enzyme negative
- Opportunistic nosocomial pathogen
 - Glucose oxidizer
 - Can acquire resistance to many antibiotics from antibiotic exposure

Stenotrophomonas maltophilia

- Rapid maltose oxidizer
- Gram negative bacillus
- Gun metal gray pigment
- Intrinsically resistant to many antibiotics
- Nosocomial pathogen super-colonizer after long term carbapenem therapy due to intrinsic resistance to the carbapenem antibiotics (Imipenem and Meropenem)







Pseudomonas aeruginosa

- Fluorescent blue-green pigment produced (pyocyanin)
- Oxidase enzyme positive
- Grape-like odor
- Grows at 42°C
 - Ps fluorescens/putida group does not grow at 42°C
- Major pathogen of cystic fibrosis
 - Mucoid strains produced in the lung due to the production of polysaccharide capsule
 - Major lung damage results from co-infection with *Burkholderia* cepacia
- Nosocomial pathogen associated with exposure to water and moist environments
- Intrinsically resistant to many antibiotics







- Chryseobacterium (Elizabethkingia) meningosepticum
 - Infections:
 - Newborns: fatal meningitis and septicemia in the newborn
 - Elderly/immune suppressed: bacteremia
 - Environmental organism found in water
 - Yellow colony,
 - Oxidase and Indole positive
- Haemophilus ducreyi
 - Cause of venereal disease: Chancroid
 - Painful necrotizing genital ulcers/inguinal lymphadenopathy
 - School of fish appearance on Gram stain from lesion
 - Requires hemin (X factor) to grow on solid media







Haemophilus influenzae

- Transmission close contact/secretions
- Virulence factor capsular polysaccharide
- Small pleomorphic Gram negative rod
- Requires 2 nutritional factors for growth:
 - X factor = hemin
 - V factor = NAD (nicotinamide adenine dinucleotide)
 - Grows on chocolate agar (contains X and V factor)
 - Will not grow on 5% sheep's blood agar
- Requires 5-8% CO₂ for growth
- Effective vaccine targets invasive infections with H. influenzae type B (HIB) effectively eliminating most childhood invasive infections
- Ampicillin resistance from beta lactamase enzyme productions [25 %], 3rd generation Cephalosporin becomes the antibiotic of choice (Ceftriaxone) for invasive infections



Haemophilus influenzae infections





Satellite phenomenon Staph aureus supplies the X and V factors required

HACEK organisms



- Oral flora organisms. Due to poor detention or invasive dental procedures organisms are introduced into bloodstream and infect heart valves
 - Fastidious Gram negative coccobacilli / need >=48 hrs to grow in culture
 - Cause of 5 -10% of community acquired native valve endocarditis not related to IV drug use
- Haemophilus species
- Aggregatibacter (Actinobacillus)
- Cardiobacterium hominis
- Eikinella corrodens
- Kingella kingii

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oxidase (-) catalase (-)
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oxidase (-) catalase (+)
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oxidase (+)
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oxidase (+), pits agar, bleach odor
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oxidase (+), hemolytic on blood agar

• Major cause of septic joint infection in small children

Bordetella pertussis

- Whooping cough 3 disease stages
 - Prodromal flu like disease most contagious stage
 - Catarrhal classic whoop cough in small children Toxin adheres to bronchial epithelial cells and cough continues until toxin wears off
 - Paroxysmal recovery phase
- Human pathogen, inhabiting nasopharynx
- Peripheral blood in whooping cough Lymphocytosis with atypical, large, irregular and deeply basophilic lymphocytes
- Tiny Gram negative coccobacillus
- Selective media = Regan Lowe Charcoal, growth in 3-5 days
- Molecular detection is standard of practice / greater sensitivity
- Reservoir for infection is in young adults due to waning immunity. Reason for booster shots in young adults







Pasteurella multocida/canis

- Normal flora in many animals (zoonotic)
 - Bite wound infections from cats and dogs
 - Human pneumonia from close contact with animals
- Small Gram negative coccobacilli
- Growth on 5% Sheep's blood agar
 - Non hemolytic grey colony
 - No growth on MacConkey agar
- Oxidase positive
- One of very few GNRs sensitive to penicillin





Capnocytophaga species

- Fusiform shaped Gram negative rods
- Fingerlike projections from colonies "Gliding"
- Dependence incubation in CO2 for growth
- Oxidase negative
- Catalase negative
- Normal mouth flora in humans and animals
- C. canimorsus infected dog bites high % of bite infections lead to bacteremia and endocarditis
- Many Capnocytophaga species in humans, normal flora
 - Infect mouth ulcers induced by chemotherapy
 - Can lead to bacteremia









Brucella species

- Brucellosis, FUO, significant joint pain, intracellular pathogen of the Reticuloendothelial system
- Specimens: Blood and Bone Marrow
 - Automated Blood culture systems with growth @ 5 -6 days
- Serology can assist with diagnosis of chronic disease
- Culture: Small Gram negative coccobacilli, non hemolytic gray colony,
 - Requires 5 10% CO2 to grow
 - Oxidase positive,
 - Urease enzyme positive
- Zoonosis Infection from ingestion of raw milk, animal exposure, inhalation
 - **B. abortus** raw cow milk
 - **B. melitensis** raw goat milk, feta cheese
 - **B. suis** pigs
 - B. canis dogs









Campylobacter spp.

- C. jejuni Diarrhea, common cause in US, bacteremia in HIV and immune suppressed
 - Ingestion of undercooked poultry / juice contaminating raw food
 - Sea gull shaped, poorly staining Gram negative rod
 - Culture requires selective blood agar containing antibiotics
 - Campy-BAP, Skirrow's BAP
 - Incubate at 42°C in microaerophilic atmosphere (high CO₂, low O₂)
 - Sequelae Significant % Guillain-Barre syndrome
- C. fetus Bacteremia in the immune suppressed host.
 - Source cattle and sheep.
- Temperature tolerance aids in identification

C. jejuni – grows at 37°C and 42°C,

C. fetus - grows at 37°C and 25°C

hippurate hydrolysis positive

hippurate hydrolysis negative





Francisella tularensis

• Reservoir – rabbits, rodents, ticks and flies



- Humans infected by insect bites or from exposure to animal blood (such as skinning rabbits with bare hands)
- Bacteria can penetrate small breaks in skin:
 - Painful skin lesions
 - Enlarged lymph nodes
 - Leads to bacteremia (ulceroglandular tularemia)
 - Pneumonia
- Small Gram negative rod/ oxidase negative
- Requires cysteine in culture medium for growth







Helicobacter pylori

- Acute gastritis with small % progressing to gastric adenocarcinoma
- Human to human transmission/ fecal oral route, poor hygiene
- Rapid and strong urease enzyme produced by this organism
 - Can be used for direct detection from gastric antrum biopsy tissue
- Small curved Gram negative bacilli
- Difficult to grow in culture
- Stool antigen for diagnosis and test of cure
- Serum antibody detection obsolete not clinically useful
- Organism stained by silver stains in gastric biopsies
- Treatment Antibiotics and stomach acid suppression





Immunohistochemical stain of gastric biopsy



Legionella pneumophila



- Pulmonary disease from exposure to water
- Requires cysteine in culture medium
 - Selective medium: Buffered Charcoal Yeast Extract agar with growth 3-5 days
- Will not stain with usual Gram stain counterstain safranin
 - Will stain with Counterstain carbol fuchsin
- Silver impregnation stains best to stain fixed tissue
- Diagnosis: Urinary antigen test (EIA) detects Legionella pneumophila type I pulmonary infection
- Serology available but seldom used for diagnosis
- Treatment: Erythromycin (macrolide)







Bacteria without cell walls

- Mycoplasma and Ureaplasma species have only cell membranes. Media for culture must contain sterols. Molecular amplification is current diagnostic method of choice.
- Lack of peptidoglycan cell wall, so will not stain on Gram stain, no colonies will be produced on agar, and cannot be treated with antibiotics that act by inhibiting cell wall formation
- M. pneumoniae
 - Community acquired pneumonia
 - Presence of high titer cold agglutinins
- Genital mycoplasmas





Mycoplasma hominis

Ureaplasma

- M. hominis form fried egg appearance on sterol containing agar
 - Can cause vaginitis, cervicitis, postpartum sepsis, neonatal infections, pre rupture of membranes
- Ureaplasma urealyticum form Dark metal-like appearance on sterol containing media
- Rapid urea hydrolysis in broth
- Non-gonococcal urethritis, upper genital tract infection, spontaneous abortion, and neonatal infections

Unusual and Difficult to Grow Bacteria

- Bartonella henselae
 - Cat scratch disease from exposure to cat and cat excrement
 - Bacillary angiomatosis vascular skin lesions +/- invasion, associated with HIV
- Bartonella quintana cause of trench fever/vector is the body louse

- Chlamydia trachomatis Serovars L1,L2,& L3
 Lymphogranuloma venereum STD that involves
 lymphatics and lymph nodes
- Chlamydia pneumoniae (TWAR agent)- Pneumonia
- Chlamydia psittaci- psittacosis, pneumonia, exotic parrot exposure







Unusual and Difficult to Grow Bacteria

- Klebsiella (Calymmatobacterium) granulomatis
 - Granuloma inguinale STD
 - Infection leads to ulcerative genital lesions
- Streptobacillus moniliformis
 - Rat bite fever or Haverhill fever
 - Infection from rat bite
 - Cell wall deficient bacteria known as L form
 - Inhibited by SPS in blood culture media and requires serum supplementation to grow
- Tropheryma whipplei
 - Whipple's disease
 - Gram positive rod (Actinomycete) distant relative of Mycobacterium avium and M. paratuberculosis
 - Found in soil and farm animals
 - Causes a diarrhea which can lead to malabsorption
 - Characteristic findings in fixed tissue





Foamy macrophages in the lamina propria

Unusual and Difficult to Grow Bacteria

- Ehrlichiosis infection caused by a Rickettsia bacteria
 - Zoonotic infection, intracellular pathogen
 - Vector is the Ixodes tick (hard tick)
 - Two genera cause infection
 - Anaplasma spp, inclusion (morula) in the PMN
 - Ehrlichia spp inclusion in the monocyte
 - Fever, leukopenia, thrombocytopenia,
 - Elevated serum aminotransferases,
 - Ehrlichiosis has no rash, which differs it from Rocky Mountain Spotted Fever caused by *Rickettsia rickettsia* which is known for rash
- Found in south central, southeast , and midwest US
- PCR, serology, and examination of blood smear for diagnosis





Spirochetes

- Borrelia burgdorferi Lyme's disease
 - Primarily found in NE part of US
 - Vector = Ixodes tick (hard tick)
 - Acute disease: Fatigue, headache, fever, and rash
 - Can progress to a chronic disease
 - Diagnosis: Serology and PCR
- Borrelia species Tick borne relapsing fever
 - Western United States
 - High fever (relapsing) with thrombocytopenia
 - Muscle and joint aches
 - Vector: Soft tick (Ornithorodos hermsi)
 - Diagnosis: Blood smear observe spirochete





Spirochete Infections

- Treponema pallidum
 - Syphilis
 - RPR and VDRL tests for antibody detection
 - Antibody algorithms for diagnosis
 - Years past: Darkfield examination to view spirochetes
- Brachyspira
 - Intestinal spirochete found on the brush border of the intestine
 - ?? Role in disease
- Leptospira interrogans / Leptospirosis
 - Fever with rash and renal involvement
 - Urine from rats and other animals contaminate water supplies
 - (1) Leptospirosis Shepherd's crook shaped spirochete(2) Presence of spirochete in a renal tubule





Darkfield from chancre lesion





Bacterial vaginosis

- Mixed anaerobic/aerobic bacterial infection
 - Rather benign infection except in pregnancy
 - Discharge: Fish-like odor and alkaline pH >=4.5
 - Usual NF organism of Lactobacillus is overgrown by:
 - Gardnerella vaginalis (aerobic Gram variable rod) primary bacterial marker for infection
 - Mobiluncus (anaerobic curved Gram negative rod)
 - Clue Cells are diagnostic for vaginosis and provides a more specific indication of infection than growth in culture
 - Molecular probe assays and amplification assays available as part of "women's health" screening panels



Anaerobic Bacteria

- Anaerobic infections can occur in virtually any organ or region of the body
 - Most are polymicrobial –with both aerobic and anaerobic species
 - Endogenous organisms (normal flora) cause infection
 - Increase in numbers due to trauma, vascular or tissue necrosis cutting off the oxygen supply to the involved tissue
- Treatment: Surgery to restore oxygen and remove necrotic tissue plus antimicrobial therapy
- Specimen collection for culture
 - Gel containing swab
 - ESwab
 - Evacuated vials (port o cult)/ oxygen free vials for fluid
 - Do not refrigerate specimen for it will cause the greater absorption of oxygen in cold temperatures and will more rapidly kill anaerobes



Anaerobic culture

- **PRAS media** pre reduced anaerobically sterile
 - Media packaged in oxygen free environment
- Most common anaerobic culture media
 - CDC anaerobic enriched blood agar
 - Kanamycin-vancomycin blood agar
 - Bile esculin agar
 - Thioglycollate broth
 - Chopped meat glucose broth
- Anaerobic chambers perform culture work in an oxygen free closed cabinet
- Anaerobic gas pack jars for anaerobic incubation of culture plates
 - Wet pack add 10 ml water to hydrogen and CO2 generating envelope/ requires palladium coated catalysts to generate heat
 - Dry pack (Anaeropack) absorbs O2 and generates CO2











Bacteroides fragilis group

Pleomorphic irregular staining Gram negative rod

- Normal flora in the GI tract
- Grow in the presence of bile
- Growth on bile esculin media and turns media black
- Resistant to Penicillin and Kanamycin
- Infections: Related to the bowel such as GI abscess
- B. fragilis group organisms
 - B. fragilis most common species
 - B. ovatus
 - B. thetaiotamicron (indole reaction positive)
 - B. uniformis
 - B. vulgatus







Growth on bile/esculin media Black pigment from Esculin production

Prevotella and Prophyromonas

- Pleomorphic Gram negative rod
- Normal flora in the upper respiratory tract
- Infections: respiratory tract abscesses
- Will not grow in the presence of bile
- Will not turn black on bile esculin media
- Unique brick red fluorescence when put under UV light
- Black pigment formed on blood agar after one week of incubation







Fusobacterium spp.

• *F. nucleatum* Long thin gram negative

bacilli – spindle shaped with pointed ends

- Normal flora upper respiratory tract
- Infections: mouth and respiratory tract and liver abscess
 - Vincent's angina necrotizing oral co-infection caused by Fusobacterium species and spirochetes
- *F. necrophorum* pleomorphic gram negative bacilli that filaments or chains of rods
 - Lemierre's syndrome oropharyngeal infection, leads to thrombosis in jugular vein, septicemia, with high fatality rate







Clostridium species

- Gram positive bacilli (boxcar shaped) form spores
 - May easily over-decolorize and appear reddish
- Clostridium perfringens



- Infections: Food poisoning, necrotic tissue abscess (Clostridial myonecrosis), bacteremia, cholecystitis
- Most common anaerobic Gram positive rod in intestine
- Important reactions:
 - Double zone of beta hemolysis on BAP produced
 - Lecithinase produced on egg yolk agar (clouds the agar)
 - Reverse camp test positive







• Clostridium botulinum – Botulism

- Adult disease preformed heat labile neurotoxin ingested, usually mass produced or in home-canned foods
- Infant disease spore ingested from nature or product of nature such as honey or household dust, spore germinates in gut producing neurotoxin
 - Begins with constipation and difficult sucking bottle
- Both forms are life threatening neuroparalytic diseases

• Clostridium tetani - Tetanus

- Gram stained cells appear like Tennis racket from terminal spore
- Infection begins with penetrating skin injury with introduction of the Tetanospasmin toxin
- Spastic contractions of voluntary muscles, hyper-reflexia, lock jaw (trismus)
- Immunization to prevent





- C. septicum
 - Bacteremia or gas gangrene in patient with underlying malignancy
 - Hematogenous spread from GI tract no trauma necessary
- Clostridioides (Clostridium) difficile
 - Disease: antibiotic associated colitis, pseudomembranous colitis from toxin production
 - Toxin A enterotoxin causing fluid accumulation
 - Toxin B potent cell cytotoxin, primary virulence factor (TcdB)
 - Binary toxin Nap1 strain produces large amount of toxin
 - Diagnosis of infection:
 - EIA methods [toxin A/B] are insensitive but detect presence of active toxin
 - Molecular methods are sensitive but only detect toxin genes
 - Selective medium Cycloserine, Cefoxitin, Fructose Agar [CCFA] culture usually only for research purposes





Actinomyces

- Branching gram positive bacilli
 - Do not form spores
 - Aerotolerant, but grow best in anaerobic conditions
- Normal flora oral, GI, vagina, skin
- Infections: oral/facial (lumpy jaw), respiratory and GI
- Can form sulfur granules in tissue
- Actinomyces israelii associated with oral, thoracic, and abdominal infections, IUD infections Bread crumb colonies in broth Penicillin susceptible.







Branching Gram positive rods of Actinomyces – antler like

Molar tooth colony



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Propionibacterium acnes (Cutibacterium acnes)

- Pleomorphic Gram positive rod
- Catalase positive,
- Indole positive
- Normal flora skin, oral, GU and GI
- Contaminate in blood cultures from skin contamination
- Pathogen of acne vulgaris
- Opportunistic pathogen: cerebral shunt infections and other endovascular and neurosurgical infections
- Firmly established as significant cause of prosthetic joint infection particularly shoulder joints
- Cultures should be held up 7-14 days
- Therapy Ampicillin

