General Principles of Microbiology

micro = small
bio = life
logy = study (of) or science
Immunology = study of our protection from foreign macromolecules or invading organisms and our responses to them

Different Classes of Organisms

Viruses / chlamydia (grow only in living cells)

- viruses cant survive & multiply on their own
- need a living host cell to carry out functions
- Nucleic acid (either RNA or DNA...never both!)
- Surrounded by protein shell (capsid)
- Attach, inject nucleic acid (penetration), highjack synthetic processes inside cells to make more viruses, package, get out while going is good

Mycoplasma (grow on non-living media)

- the bacteria that do not have a rigid cell wall and are more fragile

Bacteria (no separate nucleus; unicellular)

- bacteria are single cells (prokaryotes)
- Small (0.75 – 1.25 µm in diameter/width)
- Higher surface area / volume ratio
- have both DNA & RNA (bigger and more complicated); circular chromosome
- Binary fission (don't need sex partner; fast; replication rate (~20 minutes))
- no nucleus/ nuc membrane (nucleoid)
- rigid cell wall to keep things in place
- single bacterial cells are microscopic
- bacterial colony > millions of bacteria
- bacteria are neutral, harmful, or beneficial to our bodies
- can be used for biotech, prevent spoilage, bioremediation (breaking down toxic substances into non toxic substances, nutritional foods (eg yogurt, cheese)
- we cant live w/o bacteria

Shapes of Bacteria

- Bacteria are usually arranged in specific patterns:
  - single cells (spiral and/or rod shaped)
  - diplococci (pairs) – single plane
  - chain (divide in one plane and remain attached)
  - tetrads (cocci dividing at right angle to first plane of division)
  - division in three planes (grapelike clusters)
  - cubical packet of 8 cells (sarcinae)

Parasites

- yeast & parasite cells are eukaryotes

Eukaryotes

- Unicellular and multicellular animals and plants
- Genetic material is organized into a nucleus

Normal flora
- where the bacteria live in peace
- skin is not the best place for bacteria >> sweat inhibits bacteria, skin dry, and cold sometimes
- norma flora bacteria population:
  - greatest in the large intestine, some in the mouth
  - GI-tract: colon is inhabited by anaerobes and coliform mouth ecoli in female genital tract
  - Skin: mostly coagulase negative staphylococci
- no bacteria in the CNS or the blood; respiratory sys tries to get rid of bacteria
- normal flora protect you against disease—> out compete pathogens from the outside and prevent them from colonizing and causing disease
- no normal flora = prone to infection eg babies (babies develop NF as the grow & get exposed) & elderly have minimal normal flora thus are more prone to infection
- normal flora help with metabolism (vitamin K), immune stimulation

Transient NF
- ones u pick up everyday (eating, touching things)
- bacteria don't survive or multiple for long > body gets rid of it (immune sys) b/c its not a suitable environment

Resident NF
- transient develops in resident over time
- normal flora bacterial are usually in the deeper layers of the skin
  - when u wash ur hands > transient NF are removed; dead skin cells are removed from hands exposing resident NF> explains why post wash plate has more bacteria
  - doctors must wash hands for 20-30 seconds > why ? NF is only helpful when it is in the correct area
  - eg. if ecoli from the intestine enters the urinary tract it will cause a nasty uti
- NF is generally similar in humans
- hand sanzitiers is not an effect replacement for hand washing > why? alcohol based > drys out skin > cracks in skin > exposing skin to infection

- urine is sterile (inside the body)
- pathogen is any bacteria/ w.e that has the ability to cause disease

Body Barriers
Mechanical
- skin prevents bacteria/ viruses from entering the body > acidic, cold, dry, sweat (antibacterial)
- mucous membranes, saliva, tears > inhibitory substances that kill bacteria > when eyes are dry (no tears) you are more prone to infection
- hair traps pathogens, sweep them away

I*mmune System*
- antibodies
- complement- plasma proteins
- immune cells (T-cells, NK cells, macrophages)
- immune system (cell mediated; humoural)

How do pathogens get in ?

Adherence

- bacteria need to adhere, evade & invade the host
- bacteria needs to be “sticky” making it more pathogenic and inc ability to colonize
- what contributes to “stickiness” > surface structures (pilli, fimbriae)—the hairier the bacteria is , the more likely it is to hang out
- ligands (molecules) on bacteria create binding sites
- Capsules—>protection/barrier eg against mucous, acid
- Enzymes—> neutralization of antibodies from host etc

Toxin production (destroys some of our defences)

- Toxins are substances (usually proteins) secreted by bacteria with the hope to cause damage
- the more toxins a bacteria produces, the more damage a bacteria can cause

Exotoxins

- toxins actively excreted by LIVING bacteria cells (need energy, so cells need to be alive)
- specific affinities > can only act on certain cells; eg exotoxins for liver cells cant harm heart cells
- thermolabile > bacteria are normally protein > sensitive to heat & heat change > high temps denature proteins and kill bacteria
- potent—live active toxins & right part of body can cause severe symptoms
    - aerobic bacteria > bacteria that needs oxygen to function; majority of pathogens that cause disease
    - anaerobic bacteria > don’t like oxygen, can easily get killed by oxygen; found deep in soil
    - bacterial spores > round cell w/ a thick cell wall; protects bacteria from outside environment; uncommon; inc ability to survive in nasty environment; wall can keep out nutrients; spore is known as the dormant form of a bacteria (when the environment remains nasty); when the environment is good, spores revert back to its actively dividing form
- use antibodies to combat exotoxins

Endotoxins

- liberated when cell wall disintegrates
- bacteria cant afford to get rid of endotoxin or the bacterial cells will die
- only exposed to endotoxins when the cells lyses or dies
- less specific (no specific binding sites), cause fever, malaise, endotoxic shock (kill bacteria in a quick time > lots of endotoxins released making u sick)
- thermostable
- less potent than exotoxins

Opportunism

- compromised host from previous ailment, stress, surgery, age, race etc
- get sick more often in winter when studying for exams .. why? immune sys isnt working at full capacity > stressed, lack of sleep; winters > indoors, more likely to pick up bacterias (more exposure)
- sickness depends on the relationship between the host and the microbe
- bacteraemia: bacteria in the blood (seeing, no symptoms)
- septicaemia: having symptoms of infection from bacteria in the blood

Microbial disease

- Interaction between microorganisms and the host (us) is continuous battle
- They need to enter-live-multiply
- In order to enter, they need to colonize (establish and multiply) in/on body
- Clinical infection = easy to recognize; disease; damage to host
- Sub-clinical infection = hard to diagnose (no symptoms); people spread disease w/o knowing they are infected —> leads to wide spread infection (epidemic)
- contamination > when a microbe lands on an object (deposition), & doesn't multiply
- colonization > when a microbe lands on a living surface, multiplies but causes no symptoms
- infection > seeing symptoms of infection

Measuring Danger

- Pathogenicity = ability to produce disease
    - highly pathogenic = easily make u sick (like sniffle or stomach ache for a day or 2)
- Virulence = relative capacity to cause damage
    - highly virulent = easier to kill you (wont get sick all the time, but the sickness is terrible)
- pathogenic but not virulent = can cause sickness but not deadly > best option
- virulent but no pathogenic = difficult to make u sick, but enough in the appropriate area can make u very sick
- worse is a highly pathogenic and virulent organism > easily penetrate the body and make u very sick
- Opportunistic = do not normally cause disease but can do so when defence mechanism(s) breached or compromised

Pathogenesis

- A pathogenic microorganism enters your body...two things happen:
    1. Microorganism (invader) tries to multiply / invade and cause disease (2 deg event)
    2. Host tries to prevent #1
- Whether the invader wins or not is dependent on factors such as: transmission, pathogenicity, invasiveness (adherence, persistence, avoidance of immune system), and toxigenicity (ability to make toxins)
- bacteria/ viruses cant cause harm unless they get inside ur body

Transmission:

- inhalation (most common; respiratory tract cleans them out)
- ingestion (2nd most common; eg contaminated food, water > cause diarrhea, vomiting, stomach pain)
- breaking is protective barrier (eg mucous mem, skin; bacteria only need a small break in the skin in order to penetrate)
- direct deposit (traumatic injuries, gunshot, stab wound exposing deep tissue and organs > bypassed all protective barriers)

<u>IMMUNE SYSTEM</u>

What is immunity?

- Immunity = the protection against infectious disease conferred either by the immune response generated by immunization or previous infection or by other non-immunologic factors...a.k.a. body's ability to resist infection
- 2 types:

N*on Specific (innate) immunity:*
- simple; keeps out everything (all invaders); doesn't do anything special
- skin > acid mantle
- mucous membranes > cilia in respiratory tract, tears, pH, lysosomes
- Iron-binding proteins > some bacteria require iron for growth – Transferrin,lactoferrin
- Phagocytosis > PMNs-neutrophiles, monocytes and macrophages
  - antipahgocytosis: bacteria capsule protects it from phagocytosis
  - opsonization: host creates antibodies to alter the capsule of bacteria thus making it able to be phagocytized
- complement > complex system of plasma proteins that work together to resist bacterial infection & to enhance the immune response

Specific (adaptive, acquired)
- targets one type of invader at a time, dependant on past exposure
- 2 types:

  Humoral
  - good for bacterial/fungal infections
  - circulating antibodies
  - Antibody: protein that binds specifically to a substance (its antigen)
    - Igs or immunoglobulins that reacts specifically w/ an antigen
    - Produced by B-lymphocytes/cells upon stimulation from antigen presenting T-cells
    - Recognize toxins, capsules, some viral proteins from "self"
  - Antigen
    - "non-self"
    - anything that binds to an antibogy: Protein, glycoprotein, lipoprotein, polysaccharide
    - What structures could be "antigenic" in a bacteria? capsule, cell wall, flagella; Virus? polypeptide antigen
    - antibody binding is really specific > only one antibody can bind to a specific antigen

  Cell mediated
  - good for viral/parasite infections (intracellular organisms), tumors and other foreign cells (i.e., transplants, grafts)
  - Immune-suppressive medication for transplant recipients
  - exposure to antigen induces response from trained T- cells
  - thymus makes T-cells, not antibodies—> Helper, suppressive, cytotoxic (killer) generated from memory T-cells
  - T-cells very specific at recognizing a specific antigen and destroying it
  - T-cells can recognize infected cell & kills the cell that is harbouring the viruses/ parasites > once viruses/ parasites are released from destroyed cell the humoral response takes over
    - cells infected by bacteria & fungi (free living) = ok b/c antibodies can still cross link
    - parasite & viruses are intracellular > need to be inside a host cell > bad b/c antibodies take long to inc in # and get to where the infection is happening, thus the viruses has already been successful in taking over a cell thus, antibodies have nothing to bind to
    - T-cells finish the job antibodies cant do

Antibody Binding

- antibodies (Igs) have a constant & variable region; variable region responsible for antigen recognition
- classes of Igs
  - IgG: host defense; maternal- crosses placenta and protects newborn; y-shape
  - IgD: Role is unknown
  - IgA: host defense; found in secretions (Tears, saliva, milk, respiratory, GI and genito-urinary tract); dimer; more efficient because it has more prongs to do more cross linking
  - IgM: host defense; early immune response; pentamer
  - IgE: Hypersensitivity (allergies); defends against parasites

Immune Response

- any foreign substance will create a immune response

Primary response

- Ab production the first time you get exposed to antigen
- latent period (5-10 days) b-cells take time to become effective > bacteria take advantage and multiply quickly
- circulating antibody detectable after 5-10 days
- a week or so later bacteria are destroyed
- antibodies are still present in the body
- Antibody in serum is maximum at ~21 days, then drops to low levels

Secondary Response

- Basis for Immunizations
- Occurs when Ab is introduced 2nd, 3rd,4th ...time
- Lag, rapid Ab increase (2-3 days), slow decrease
- Booster injections to maximize Ab levels
- immunity > b-cell is primed, no longer need latent period, immediate protection > very low chance of becoming sick again

Antibody Detection

Serological Reaction

- Detects presence of antibodies in serum sample – Antigen and antibody interact; agglutination
- Antibody titration: quantitation of antibody in patient sera
- Detect unknown microorganisms using known antisera
- if u have A antigens > u have A blood etc

Disorders of Immunity

1 Allergy and Hypersensitivity

- OVER-reaction to antigens in absence of true infection
- Can be fatal…..ANAPHYLAXIS
- diet has change (access to more & different types of food) body is exposed to many more antigens > becomes over worked > messes up immune response > extra sensitivity reaction > hypothesis is not proven

2 Auto-immune diseases

- Immune system reacts to its own “self” antigens
- “auto-antibodies”

- Type I diabetes, MS, rheumatoid arthritis, lupus

3 Immunodeficiency states
- Inability to produce antibodies and/or dysfunctional CMI
- Congenital, disease, AIDS
- chemo can also reduce the efficiency of the immune sys
- isolate a patient from being expose to the pathogen

4 Graft rejection
- NORMAL immune reaction to “non-self”
- Control by immune-suppressive medication
- find the balance b/w lowering the immune sys & not exposing the patient to serious infection

Bacterial Infections
- easily treated by antibiotics

Viral Infection
- antivirals are expensive, and hard to come across
- they also cause nasty side effects
- work to on preventing, rather than treating viruses

Immunization
Passive Immunization
- administration of pre-formed antibody IgG against a specific microbial agent
  - pre-make antibodies to neutralize the toxins to help the patient gets better before the patients body makes its own
  - this is expensive and difficult
- IgG animal origin: short lived disappear after 10 days, risk of hypersensitivity reaction; usu from horses
- IgG human origin: short lived disappear after several weeks; no risk of reaction
- Gamma globulin: pooled from large grouped of blood donors and has antibodies to many common infections
- Hyperimmune globulins: specific for a particular microbe
- neuro toxins need be treated asap eg rabies or it causes rapid permanent damage to the CNS

Active Immunization
- trigger a immune response using a vaccine (antigen)
- longer lasting

- *Live-attenuated vaccine*
  - Sub-clinical or mild illness mimicking the disease
  - Local (IgA) and humoral (IgG) immunity (life long lasting)
  - Rapid immunity development
  - Serious illness in immuno-compromised individuals (small risk)

- *Killed vaccines subunit toxoids*
  - Antigens without infectivity
  - May require boosters
  - Adjuvant with toxoids

- Polysaccharide vaccines can be conjugated to protein (see conjugate vaccines)
- dead or a small part of the pathogen (missing components eg the ability to multiply) can trigger reaction but not the disease; much safer but the protection is not as strong

- *Recombinant vaccines*
  - very specific, clean & extremely safe; can mass produce protein > cheap & effective
  - DNA recombinant technology; virus doesn't survive inactivation process
  - Attenuates microorganism
  - Hep B vaccine
- *Absorbed vaccines*
  - Vaccine mixed with inorganic salt for slower adsorption and longer- lasting immunity
  - Tetanus, diptheria
- *Conjuagte vaccine*
  - Designed for poorly antigenic microorganisms
  - Conjugate antigen of interest to immunogenic, non-toxic protein
  - adding 2 proteins tg; difficult to do and expensive
  - Haemophilus influenzae type b
- *Combined vaccine*
  - mixed 4 diff proteins from diff bacteria into one vaccine > multiple protection in one vaccination shot eg EPT, MMR; made possible b.c of recombinant vaccines
  - for ease of administration

- *Combined active-passive immunization*
  - Immediate protection after possible exposure to microbe
  - Hyperimmune Igs and vaccine injected at DIFFERENT sites
  - Tetanus, Rabies, Hep B

Antibiotic Resistance
- The first antibiotic penicillin discovered in 1929 by Sir Alexander Fleming
- World War II penicillin used to treat staphylococci and streptococci (1946)
- Resistance to penicillin recognized almost immediately
  - 80% of all strains of Staphylococcus aureus
  - Streptococcus pyogenes (Group A strep) still treated with penicillin
  - Interestingly, penicillin has never been effective against Gm-negatives (Salmonella, Shigella, Bordetella pertussis, Yersinia pestis, Pseudomonas)
- penicillin is a broad spectrum antibiotic > kills off many pathogens
- penicillin is highly overused > antibiotics are very safe b/c for an antibiotic to work, it must bind to a cell & cells that have binding sites for antibiotics are bacteria thus they don't attack the human cell
- antibiotics are only effective against bacterial infections
- every living cell has the ability to mutate > mutation can change a binding site & make the bacteria cell resistant to the antibiotic (penicillin)
  - thus when you take antibiotics it will kill all the bacterium but the bacteria that is resistant will remain > later it will start to replicate and you have multiple antibiotic bacterium
  - this is known as mutation with selective pressure

Antibiotic Therapy

- Effective chemotherapy depends on selective toxicity—> good against pathogen, does not affect host
- Exploit pathogen processes not seen in humans eg cell wall, metabolism, etc.
- Knowledge of likely microorganism is crucial.. site? organism? allergy to host?
- Other considerations... route of administration; Monitoring therapy; Adverse effects on GI-tract, skin, haemopoietic system, renal system, liver
- anytime we use an anti biotic we create (selective) pressure > now doctors are encouraged to test patients first before prescribing antibiotics
- monitor if the antibiotic is working

Acquired Resistance

- Resistance occurs when a susceptible microorganism is no longer inhibited by an antibiotic
- 3 major mechanisms of resistance:

Alteration in drug target

- most common
- a mutation changes the binding site > antibiotic does work anymore
- eg cell wall proteins, enzyme, ribosome

Production of inactivating enzymes

- produce enzymes to destroy the antibiotic before it reaches the bacteria

Decreased uptake of antibiotic

- eg cell wall change so antibiotic cant enter the cell
- cell pumps antibiotic out of the cell and thus it cant kill the bacteria inside (efflux)
- **** get examples from textbook lmaoooo ?*** eg β -lactams, chloramphenicol

- Intrinsic vs Acquired Resistance
  - *intrinsic:*
    - characteristics of microorganism
    - antibiotic's mechanism of action (inherent or "natural")
    - predictable > u know antibiotic wont work against an organism b/c its missing a protein > eg prescribing the wrong antibiotic
  - *acquired:*
    - new or added (driven by two genetic processes in bacteria...mutation and selection (vertical evolution); and exchange of genetic material (horizontal evolution)
    - treatment fails because the bacteria has unexpectedly changed and become resistant

- Mutations lead to
  - Change it site of antibiotic target (but protein for bacterial still works fine!)
  - Regulatory genes
    - turn on alternative path
    - turn on efflux mechanisms
  - Change cell permeability

Post antibiotic era:

- we need to cut back on using antibiotics b/c we are forcing bacteria to evolve
- eg MRSA penicillin, vancomicin, and methacillin resistant organism

- farmers put antibiotics in herd feeds > led to pressure & inc in antibiotic resistance
- teething toys for babies are antibiotic treated, same as cutting boards & even underwear !

Decreasing antimicrobial resistance
- Withhold antibiotics—> for self-limited viral infections (i.e., the “common cold”)
- Use narrowest spectrum (kill only certain types) antimicrobial agents
- Base decision about broadness of empiric antibiotic coverage on severity of illness
  - clinically stable and not at risk for significant morbidity...may be appropriate to wait culture results and MIC testing
- prevent your self from getting sick > good hygiene, cooking food
- Education
  - helps to achieve therapeutic and preventative goals
  - when are antibiotics needed? how to take them?
  - proper duration—>low to high resistance bacteria thus not using long enough > kill weak bacteria but strong ones remain & reproduce creating a highly resistant population of bacterium
- Earlier detection of therapeutic failure—> good for patients with antibiotic-resistant pathogens

<u>Diagnostic Microbiology</u>
- Isolation of pure culture from specimen b/c mo exist in nature as mixed specimens
- Culture media: need to consider source of sample, suspected species & its nutritional requirements
- CSF and blood should be sterile > easy to detect infection
- stool sample have many bacteria + normal flora (ecoli) > difficult to detect infection
- inoculation method > streaking/spreading/pour > scoop specimen > spread it on a plate > incubate it and a colony will form > must isolate colony from mass (sterilize then bunsen burn that wipe the blob till it gets smaller and smaller)
- Pour/spread plates—Bacteria grow inside agar; Used for enumeration of bacteria

Preservation of Cultures
- we keep a copy of bacteria from a patient eg for research purposes
- short-term: stored in medium refrigeration temperatures (4 to 10oC
- long-term: freeze liquid nitrogen (-195oC), freezers (-80oC), lyophilization (freeze-drying; dehydration then vacuum seal)

Identification
- we can see colonies b/c them are a clump of 10 mil bacteria
- need a microscope to see a cellular morphology
- colonial morphology is not enough to make a diagnoses ***DO I NEED TO KNOW SHAPE**
- resolving power (resolution) = ability to distinguish two closely located objects as separate, distinct entities

Staining Techniques
- staining helps you find bacteria; 3 steps
  1. Smear bacteria onto a glass slide
  2. fix bacteria to slide by heating (w/ a bunsen burn) bacteria will then bind to the slide
  3. stain w/ desired dye

Simple vs differential stains

Simple

- single dye normally used
- all organisms same colour
- look at size, shape, number, arrangement, etc

Differential stains

- two or more dyes
- diff bacteria stain diff colours; more informative & more useful
- differences between microorganisms or parts of cells
- eg acid fast, Gram (most common)

Gram Stain

1. Flood slide with crystal (or gentian) violet. (Wash with running tap water).
2. Flood with Gram's iodine (help stain bind tighter) then Wash with water
3. Carefully decolourize with 95% ethanol for 20 sec then wash with water…20 sec is enough to remove the stain from the thin layer but not enough to remove from the thick gram +ve cell wall…alcohol increases permeability of Gram-negative outer membrane but shrinks pores of Gram-positive peptidoglycan…most critical step
4. Flood with safranin (pink colour). (Wash with water). Air dry, or blot with absorbent paper… pink safranin > gram -ve will take the pink, but the purple colour will hide the pink in the gram +ve cell wall

2 possible outcomes from gram stains:

- purple (gram +ve) or pink stain (gram -ve)
- 99% of bacteria can stain w/ the gram stain
- gram stains let you see individual cells > more effective testing
- cell wall important for:
    - Essential for cell growth and division
    - Shape of bacteria related to peptidoglycan layer
    - peptidoglycan layer is thick in gram +ve but thin in gram -ve
    - gram -ve has an outer membrane
    - stain binds specifically to the peptidoglycan layer (dark purple in gram +ve b/c layer is thicker)

Gram -ve Cell Envelope

- lipopolysaccharide = endotoxin on outer mem + periplasm
- only gram -ve bacteria can produce endotoxins (gram +ve doesn't have outer cell membrane)
- both gram -ve & gram +ve can produce exotoxins
- thin peptidoglycan
- dye pink

Gram +ve

- teichoic acids often found attached to peptidoglycan to give negative charge to help transport of positive ions and storage of phosphorus
- thick peptidoglycan
- dyes purple

Other Stains

- Endospore- malachite green applied with heat to penetrate spores followed by counter - staining with safranin
- Capsule- treat with copper sulfate before staining to visualize capsule as a clear zone surroundings cells
- Flagella- use of mordant to thicken flagella before staining to visualize

Fluorescence Microscopy
- dye fluoresces at specific wavelength
- antibodies tagged with dyes are common (immunofluorescence microscopy)

Electron Microscopy
- Electron beam (instead of light)
- Million times magnification possible (0.003 µm)
- TEM (stain with heavy metals); SEM (3-D image of cell surface)

Culturing Bacteria
- Chemically defined – exact composition known; use in research labs
- Chem undefined—some components can't be controlled (beef extract, blood, etc.); dont know whats in it, thus different nutrients can effect ur results; use in diagnostic labs
- If solid (versus liquid) growth – 1.5% agar used; usually use solid agar than liquid because they form colonies
- Enrichment media – increase # of specific bacteria in sample by favouring growth of interested species
- Tissue culture media – for cultivating viruses, derived of plant or animal cells (need actively dividing cells

Media Requirements
- Bacteria – requirements vary
- Yeasts – high sugar and lower pH
- Anaerobes – must remove oxygen

Selective, differential and S/D media
- Selective media – enhance growth of one bacterial species or suppression of another; ingredient that is harmful to the bacterial but is beneficial to another
- Differential media – differentiate bacteria based on their nutritional requirements and phenotypic characteristics; look different in colour size etc in order to differ one bacteria from another on an agar plate eg differ by colour or shine
- Selective / Differential media – very/most useful in clinical labs (e.g., MacConkey agar)

MacConkey

Salmonella typhimurium
Gram Negative: growth
Lactose Fermentation: negative (colorless colonies)

Escherichia coli
Gram Negative: growth
Lactose Fermentation: positive (pink colonies)

- use differential media from bile salts & crystal media (eg purple stain in the gram stain); they bind to the cell wall of gram bacteria and messes up the cell wall so that they cant multiply
- they are selective against gram +ve bacteria (cant grow on the macConkey agar plate)
- bacteria like sugar as a nutrients source to grow. the best sugar for bacteria is lactose
- bacteria break down lactose w/ lactase and release E and produce lactic acid (not all bacteria make lactase)
- thus bacteria that can use lactose will be able to grow in a macconkey plate
- a cell can only handle so much acid or it will eventually be destroyed
- thus bacteria need to use E to pump out the lactic acid —> last ingredient on agar plate is a pH indicator called phenol red (when soln is acidic, it will turn bright red)
- lactose fermenting bacteria will then produce colonies that are red in colour
- bacteria that cant ferment lactose stay a neutral or beige looking colour (cuz no pH change to create a red colour)
- ecoli is gram -ve; rod shaped (bacillus) bacteria —> pink colour in the gram stain

Temp
- psychophiles: like low temp; grow best at temperatures 15-20oC
- mesophiles: most human bacteria; b/c they grow in temp similar to out body; grow best at temperatures 25-40oC approx 37oC
- thermophiles: like hot temp; grow best at temperatures 40-85oC
- extreme thermophiles: Pyrolobus fumarii; “fire lobe of the chimney”; Lobe shape; in the walls of a deep sea hydrothermal vent; Grows between 30 and 113C; 106C is optimal

Oxygen Requirements
- some pathogens need O2, some are killed by O2
- very soft semi-solid agar tubes + nutrients
- growth at the top indicates they require oxygen —> obligate aerobes
- flaculate aerobes= most need O2
- obligate anerobes cant tolerate 02
- aeroteolerent anerobes dont care
- microaerphiles—> most difficult to culture; use O2 for E rxns; need to know what to put in the medium

pH & water requirements
- Optimal pH varies from bacteria to bacteria
- Intracellular pH must be ~7.5
- Growth observed at pH values of 4-9 (optimum 6-8)
- Water (light) can be important for certain microorganisms
- Osmotic pressure (hypertonic, hypotonic, isotonic)
- most bacteria would prefer a neutral or alkaline environment
- molds and yeasts generally have a broader pH range for growth than bacteria. Optimum pH is ~ 5 to 6.
- growing cells release acidic or alkaline waste products into the growth medium environment. Without buffering of the medium, the shift can eventually inhibit growth
- they like a damp/ wet environment
- cell wall of bacteria is not very rigid; eg is solution is hypertonic the cell can shrink and rupture; thus you need to know they salt concentration the bacteria cell can grow then find an isotonic solution proportionate to it

Gram +ve Cocci

Staphlyococcus aureus

- Genus name is capital, species name is lower care; thus names are italics or underlined
- staphlyococcus means grape like
- s. aureus is very succesful pathogen b/c is produce lots of toxins
  - cytotoxins: damage cells of the body
  - haemolysins: toxins that lysis rbc to protect itself from antibodies and use RBC iron for E
  - enterotoxin: tell u where they toxin acts on (in the enteric system); cause diarrhea, vomiting
  - diary and creamy substances usually cause this infection
  - symptoms come on quickly
  - exfoliative toxins affect the skin "scaled skin)
  - toxic shock syndrome toxin 1 (used to be exotoxin C and enterotoxin F)
- enzymes it creates:
  - coagulase:
    - (coagulation of fibrin) clumps blood; made by almost all pathogenic staphylococci; used in laboratory test to differentiate from S. epidermidis, S. capitis and S. saprophyticus; only s.aureus can produce coagulase….no other species can
  - Beta-lactamase (penicillinase):
    - destroys penicillin cant be killed by penicillin; over resistant in the hospital
- s.aureus is only true pathogen
- Many S. aureus strains are found in normal population (~15%)
- Carried in anterior nares, axilla, perineum and hands
- Problem:
  - 85-90% of strains isolated in hospital are penicillin resistant!!!
  - Localized purulent infections (pustules, boils, styes, conjunctivitis, otitis, etc.)
  - Pneumonia, osteomyelitis, septicaemia, endocarditis
  - Food poisoning, toxic shock syndrome, scalded skin syndrome
- Important cause of hospital acquired nosocomial infections from stitch abcesses, infected wounds, or generalized infections
- Preventative measures include
  - Aseptic technique in ER and OR, wound precaution
  - Education of health personnel
  - Handwashing!

S. epidermis

- not a true pathogen b/c it doesnt cause probs most of the time
- Part of normal skin/mucous membrane flora
- Non-pathogenic, except in compromised patients where can cause post-operative infections (brain, open heart, endocarditis, shunt infections)
- Considered an opportunistic pathogen

Staphylococcus saprophyticus - in the environment and on skin; can cause urinary infections

Steptococci

- Arranged in pairs or forming chains
- have many pathogens in its genus
- "streptos" - Greek word for twisted
- subdivided into "groups" based on
  - haemolytic properties (alpha, beta)

    - have halo of red around colonies —> indicate haemolyosin production
    - alpha—> green halo, partial hemolosis, rbc not completely destroyed
    - gamma—> no hemolosis
    - beta—> complete hemolosis; red halo, clear zone
  - carboydrate C antigen (Lancefield classification)
  - M-protein (hairlike structures on the bacteria —> use then to make antibodies)
    - divides beta-haemolytic; found in mostly group A
    - M-protein these test if u make antibodies to the bacteria. when testing which ever antibodies are created…you know these were the diseases

Streptococcus pyogenes

Group A, beta-hemolytic, S. pyogenes causes:
  - acute tonsillitis (strept throat) – can lead to rheumatic heart disease
  - impetigo, cellulitis, etc. (skin infections)
  - fever and septicaemia

Caused by toxins
  - streptolysins (O and S) —toxic for neutrophils and macrophages
  - streptococcal pyrogenic exotoxins (Spes)—scarlet fever rash
- enzymes it uses:
  - hyaluronase helps break down hyuronic acid which holds body structures together —> allows pathogens to spread easier
  - causes flesh eating disease (necro facitis) —> toxins due damage
    - hijacking human plasminogen from blood, attach to surface and activate it into protease...good for spreading...
    - bacteriophage has gene encoding for enzyme allowing bacteria to escape entrapment and killing by neutrophils (white blood cells)
    - need amputation; grafting helps now too
- Virtually all are penicillin G sensitive (vs. S. aureus)

Streptococcus agalactiae
- Group B
- a normal flora in the female gentile tract (can cause neonatal infections during birth)
  - early septicaemia—> respiratory distress or shock at birth; high fatality rate (serious); rare
  - delayed meningitic form—> 1-12 weeks post-partum; treat immediately or cause sequelae (ltm secondary effects)
- babies are more sustepitble —> underdeveloped immune system & they dont have normal flora to outcompete pathogens

Strep faecalis
- opportunitistic and naturally resistance to alot of antibiotics
- if taking antibiotic for another disease —> it will take over body
- Group D, aka Enterococcus
- Part of normal flora of GI-tract cause uti, meng, carditis

Viridens streptococci
- Found in oral cavity of health individuals
- Can cause endocarditis in individuals with damaged heart valves

Streptococcus pneumoniae

- Also known as pneumococcus
- diplococci
- Polysaccharide capsule has antiphagocytic properties—> ~90 distinct capsular serotypes
- Found in naso-pharynx of healthy individuals
- Can cause
- lobar pneumonia- auto infection , meningitis, bacteremia
- Prevention strategies (elderly, alcoholics, crowded living, vaccination)

GRAM -VE COCCI

eg exam question a gram +ve dipolococci ? strept auerus

Neissera. meningitidis

- Gram negative diplococci
- grown in enriched media
  - Laboratory isolation using chocolate agar, 5-10% CO_2, 37 C
  - use selective media (i.e., Thayer-Martin) when isolating from nasopharynx
  - difficult to culture
- Frequently found in the naso-pharynx of healthy individuals
- Antiphagocytic polysaccharide capsule
  - 13 different serogroups—> A, B, C, X, Y and W135 most prevalent
- Carriers can occasionally develop infection or pass organism to non- immune individuals who develop infection
- Only infects humans!!!
  - usually children or those living in crowded living quarters
  - occasional epidemics
- Infection can result in:
  - Meningitis
  - Septicaemia (starts as skin rash)
  - Waterhouse-Friderichsen Syndrome (most severe form of septicaemia by N. meningitidis)
- Penicillin is primary antibiotic used
- Vaccination is recommended for children (11-12 years), teenagers and college/university students living in dormitories
  - Conjugated vaccine for serogroups A, C, Y and W135
  - Now have meningococcus vaccine for infants at 2-5 months (serogroup C)

N. gonorrhoeae

- causes lots of sti's esp gonorrhea
- Gram negative diplococci, 0.6-1µm in diameter
- In a clinical lab, grow on Thayer-Martin plates, in damp environment
- with CO_2
  - VERY sensitive to drying and changes in temperature
- Second highest reported STD after chlamydia in US
  - >350,000 cases/year reported in the US (2001) • Number of cases is now decreasing every year
  - gonorrhea has gone down b/c people are trying to protect themselves from HVI
- Clinical gonorrhea
  - MEN: causes acute infection of urethra (90-95%)
  - WOMEN: 50% are ASYMPTOMATIC!!! —> Cervicitis
  - If untreated can cause PID, sterility

- Disseminated Gonococcal Infection (DGI)
  - 1-3% cases, usually women
  - Fever, skin infection, arthritis
- Neonatal infections
  - Rare, but newborns can acquire infection from mother during birth
  - Causes gonococcal ophthalmia neonatorum (acute purulent conjunctivitis)
- DIAGNOSIS
  - MEN: use microscopy to directly observe swabs of urethral discharge
  - WOMEN: culture is necessary from endocervical, urethral and anal swabs
- PREVENTION and TREATMENT
  - Penicillin resistance is emerging (South-East Asia, West Africa, Canada and US)
  - Treat using ceftriaxone, cefixime, ciprofloxacin or ofloxacin combined with doxycycline/ azithromycin
  - Resistance to ciproflaoxacin (quinolones) emerging
  - SIMULTANEOUS treatment of partners is ESSENTIAL
  - No vaccine available