Different classes of organisms:

- Viruses (grow only in living cells)
- Mycoplasma (grow on non-living media)
- Bacteria (no separate nucleus; unicellular)
- Parasites

- Small (microscopic)
    - 1-2 microns (1 mm = 1000 microns)

Viruses:

- Nucleic acid (either RNA or DNA… never both)
- Surrounded by protein shell (capsid)
- Enter cells and divert the synthetic processes of those cells towards their own replication
    - This can sometimes kill the host cell
- Summary: Attach, inject nucleic acid (penetration), highjack synthetic processes inside cells to make more viruses, package, then get out

Bacteria

- Prokaryotes
- Rigid cell wall to keep things in place
- Genetic material organized into a circular chromosome
- No nucleus (nucleoid)
- Both DNA and RNA
- Binary fission to reproduce
- Some bacteria do not have a rigid cell wall and are more fragile (ie. mycoplasmas)

Eukaryotes

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

Are all bacteria bad?

- No : biotechnology, spoilage of food, bioremediation (the intentional release of certain strains of bacteria - can break down toxic substances), functional foods (eg. yogurt - good bacteria), etc.

“Normal” flora (good guys)

- Resident (bacteria lands in the perfect envt and establishes in the body) vs transient (bacteria when it first enters the body; it might not be able to colonize in the body)
- GI-tract (large intestine especially): colon is inhabited by anaerobes and coliforms
- Skin: mostly coagulase negative staphylococci
- Transient flora is found in a specific location often as a result of displacement of resident flora, injury or trauma, or through human behaviour

- Where should there be no bacteria? - central nervous system, circulatory system, urine inside your body

  What can they do for us?
  - Protection from invasive (bad bacteria)
  - Metabolism (certain microorganisms synthesize vitamin K), immune system

  Mechanical barriers
  - Skin, saliva, mucous, tears, hair, etc.

  Other helpers include
  - Antibodies
  - Complement
  - Immune cells (T-cells, NK cells, macrophages)
  - Immune system (cell-mediated; humoral)

How do the bad guys get in?
- Adherence
- Toxic production (destroys some of our defenses)
- Opportunism
- Compromised host (how does this happen?)

- Bacteraemia vs septicaemia (anaemia=blood)

<u>Infectious Disease and the Human Immune Response</u>

Microbial Disease
- Interaction b/w microorganism and the host (us)
  - They need to enter/live/multiply
- In order to enter, they need to colonize (establish and multiply) in/on body
  - clinical infection (disease) can result when damage occurs to host (contamination = deposition without multiplication)
  - Clinical disease = easy to recognize
  - Subclinical infection = hard to diagnose (no symptoms)

How do we measure how dangerous it is?
- Pathogenicity: ability to produce a disease
- Virulence: relative capacity to cause damage (ie. the degree of pathogenicity)
- Opportunistic: do not normally cause disease but can do so when defense mechanism(s) breached or compromised

- A pathogenic microorganism enters your body… 2 things happen:
  1. Microorganism (invader) tries to multiply/invade and cause disease (2^o event)
  2. Host tries to prevent #1

- Whether the invader wins or not depends on several factors
- Transmission:
    - Inhalation, ingestion, break in protective barrier, direct deposit (severe injury causes tissue to be directly exposed)
    - Pathogenicity
    - Invasiveness (adherence, persistence, avoidance of immune system)
    - Toxigenicity (ability to make toxins)

How does a pathogen adhere to us?

- A bacteria needs to adhere, evade, and invade the host
- Tools used to achieve these huge objectives:
    - Surface structures (pili, fimbriae)
    - Capsules
    - Enzymes

Toxigenicity

- Toxins are substances (usually proteins) secreted by bacteria with the hope to cause damage
- 2 classes:
    1. Exotoxins
        a. excreted by living cells
        b. Specific affinities
        c. Thermolabile
        d. Potent
    2. Endotoxins
        a. Liberated when cell wall disintegrates
        b. Less specific, causes fever, malaise, shock
        c. Thermostable
        d. Less potent than exotoxins

January 15th, 2019

Immunity: the protection against infectious disease conferred either by the immune response generated by immunization or previous infection or by other nonimmunologic factors (aka body's ability to resist infection

2 types of immunity:

- Non-specific (innate)
- Specific (adaptive, acquired)

Innate Immunity:

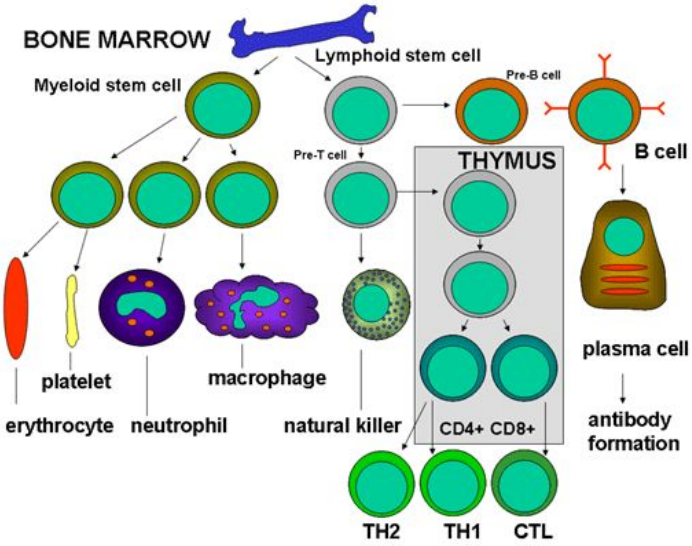
- Skin
    - Effective because it keeps out both good and bad bacteria

- Mucous membranes (mechanical)
    - Cilia in respiratory tract
    - Lysosomes, pH
- Iron-binding proteins
    - Some bacteria require iron for growth
    - Transferrin, lactoferrin
- Phagocytosis
    - PMN's, monocytes and macrophages
- Complement (endogen presenting cell?)

Specific Immunity

- Humoral and cell-mediated (CMI)
- Difference between innate immunity and adaptive immunity:
    - Innate: protects against ANY invader, does not discriminate
    - Adaptive: directed against one type of invader, dependant on past exposure

Where do immune cells come from?



Humoral Immunity

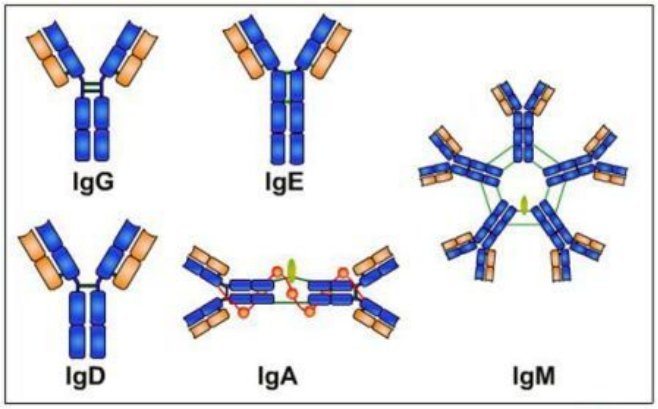
- Circulating antibodies
- Antibody: protein that binds specifically to a substance (its antigen)
    - Igs or immunoglobulins
    - Produced by B-lymphocytes upon stimulation from antigen presenting T-cells
    - Recognize toxins, capsules, some viral proteins
- Antigen

- “Non-self”
- Protein, glycoprotein, lipoprotein, polysaccharide
- What structures could be “antigenic” in a bacteria? Virus?

Antibodies are very specific
- They have a very specific bacteria that will only bind to a very specific antigen

Immunoglobulins (Igs) aka. Antibodies
- Antibody: Ig produced in response to stimulation by an antigen and reacting specifically with it
- Distinguish “non-self” from “self”
- Constant and variable region
    - Variable region is responsible for antigen recognition

Classes of Igs
- 5 classes: IgG, IgA, IgM, IgE, IgD
- IgG
    - Host defense
    - Crosses placenta and protects newborn
- IgD
    - Role is unknown
- IgA
    - Host defense
    - Found in secretions
        - Tears, saliva, milk, respiratory, GI and genitourinary tract
        - Dimer
- IgM
    - Host defense
    - Early immune response
    - Pentamer (makes it more effective for binding)
- IgE
    - Hypersensitivity (allergies)
    - Defends against parasites



<u>1° and 2° immune response</u>

1° response
- Ab production triggered on first antigen introduction
- Latent period of several days

- Circulating antibody detectable after 5-10 days
- Antibody in serum maximum at ~21 days, then drops to low levels
- (very slow process)

2° response
- Basis for immunizations
- Occurs when antigen is introduced 2nd, 3rd, 4th, etc. time
- Lag, rapid Ab increase (2-3 days), slow decrease
- Booster injections to maximize Ab levels
- (almost immediate)

Antibody Detection:

Serological reaction
- Detects presence of antibodies in serum sample
- Antigen and antibody interac; agglutination
- Antibody titration
- Detect unknown microorganisms using known antisera

Cell-Mediated Immunity (CMI)
- T-cells not antibodies!
    - Helper, suppressive, cytotoxic (killer) generated from memory T-cells
    - Exposure to antigen induces response from trained T-cells
    - Essential for defense against intracellular organisms, parasites, tumors, and other foreign cells (ie. transplants, grafts)
    - Immunosuppressive medication for transplant recipients

Disorders of Immunity:
1. Allergy and hypersensitivity
    - Over-reaction to antigens in absence of true infection
    - Can be fatal - anaphylaxis
2. Autoimmune diseases
    - Immune system reacts to its own “self” antigens
    - “Autoantibodies”
    - Type 1 diabetes, MS, rheumatoid arthritis, lupus
3. Immunodeficiency states
    - Inability to produce antibodies and/or disfunctional CMI
    - Congenital, disease, AIDS
4. Graft rejection
    - Normal immune reaction to “non-self”
    - Control by immunosuppressive medication

Immunization

Passive Immunization

- Administration of preformed antibody against a specific microbial agent
- IgG animal origin: short lived, risk of hypersensitivity reaction
- IgG human origin: short lived, no risk of reaction
- Gamma globulin (IgG): pooled from large groups of blood donors and has antibodies to many common infections
- Hyperimmune globulins (IgG): specific for a particular microbe

Active Immunization

- Stimulates immune system by administration of antigen
- Longer lasting
- Live-attenuated vaccine
    - Sub-clinical or mild illness mimicking the disease
    - Local (IgA) or humoral (IgG) immunity
    - Rapid immunity development
    - Serious illness in immunocompromised individuals
- Killed vaccines, sub-unit vaccines and toxoids
    - Antigens without infectivity
    - May require boosters
    - Adjuvant with toxoids
    - Polysaccharide vaccines can be conjugated to protein (see conjugate vaccines)
- Recombinant vaccines
    - DNA recombinant technology
    - Attenuates microorganism
    - Help B vaccine
- Adsorbed vaccines
    - Vaccine mixed with inorganic salt for slower absorption and longer-lasting immunity
    - Tetanus, diphtheria
- Conjugate vaccines
    - Designed for poorly antigenic microorganisms
    - Conjugate antigen of interest to immunogenic, non-toxic protein
    - *Haemophilus influenzae* type B
- Combined 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 by 1929 by Sir Alexander Fleming
- World War II
  - Penicillin used to treat staphylococci and streptococci (1946)
- How effective was penicillin
  - Worked really well for a while
  - But then it didn't work against certain strains of bacteria

- 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) because…
- Late 1940s and early 1950s because…

Antibiotic Therapy

- Effective chemotherapy depends on selective toxicity
  - Good against pathogen, does not affect host
- Exploit pathogen processes not seen in humans
  - Cell wall, metabolism, etc.
- Knowledge of likely microorganism is crucial
  - Site
  - Organism
  - Allergy to host?
- Other considerations…
  - Route of administration
- Monitoring therapy
- Adverse effects
  - GI-tract, skin, haemopoietic system, renal system, liver

Acquired resistance

- 3 major mechanisms of resistance
  - Alteration in drug target
  - Production of inactivating enzymes
  - Decreased uptake of antibiotic

* know a few examples from the table on p. 27 in the notes

- Resistance occurs when a susceptible microorganism is no longer inhibited by an antibiotic agent
- Many reasons why this can happen
    - **Intrinsic**: characteristics of microorganism vis-a-vis antibiotics mechanism of action (inherent or "natural")
    - **Acquired**: new or added (driven by 2 genetic processes in bacteria...mutation and selection (vertical evolution); and exchange of genetic material (horizontal evolution)

The chromosome: role in antibiotic resistance

- Mutations lead to
    - Change in site of antibiotic target (but protein for bacteria still works fine)
    - Regulatory genes
        - Turn on alternative path
        - Turn on efflux mechanisms
    - Change cell permeability

Is a post-antibiotic era possible?

- With current overuse of antibiotics, we are forcing bacteria to change (evolve) in order to survive
- How is this achieved/helped by us?
    - eg. animal agriculture

Decreasing antimicrobial resistance

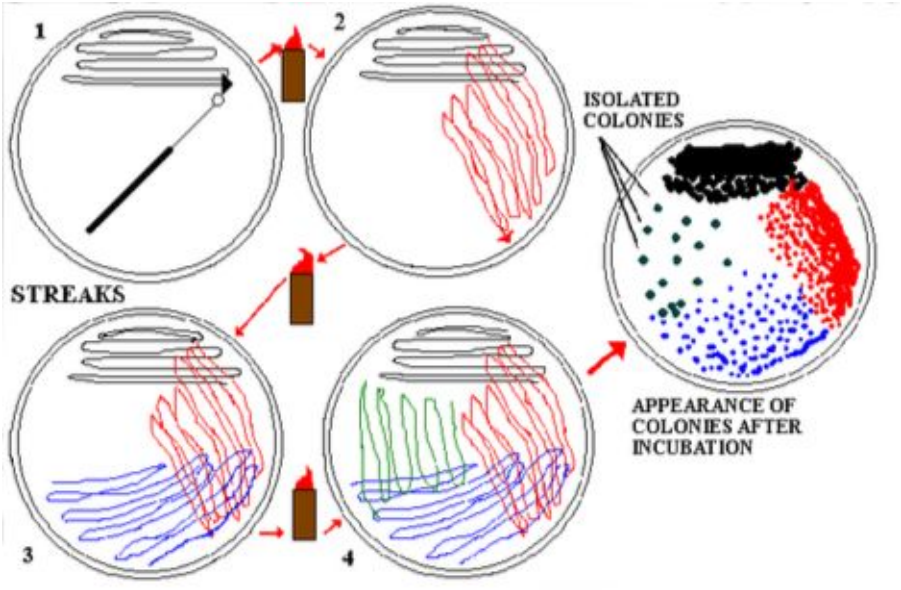
- Withhold antibiotics
    - Self-limited viral infections (ie. the common cold)
- Use narrowest spectrum antimicrobial agents
- Base decision on about broadness of empiric antibiotic coverage on severity of illness
    - Clinically stable and not at risk for significant morbidity… may be appropriate to wait culture results and MIC testing
- Prevention of infection
    - Hygiene, handwashing
- Education
    - Helps to achieve therapeutic and preventative goals
    - When are antibiotics needed?
    - How to take them?
    - Proper duration!
- Earlier detection of therapeutic failure
    - Good for patients with antibiotic-resistant pathogens

Diagnostic Microbiology

- Isolation of pure culture from specimen
    - Because microorganisms in nature exist as mixed cultures
- Culture media
    - Source of sample tested
    - Species suspected to be in sample
    - Nutritional requirement of the suspected organisms
- Inoculation methods
    - Streak, spread, or pour

Streaking a plate for isolated colonies

- Should be done in this way:

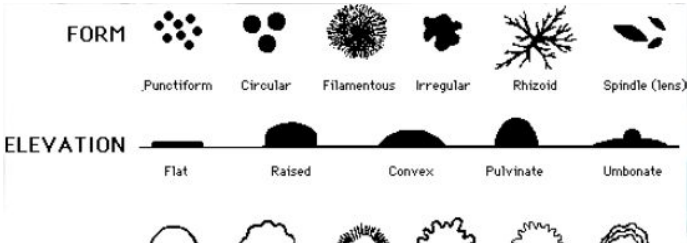


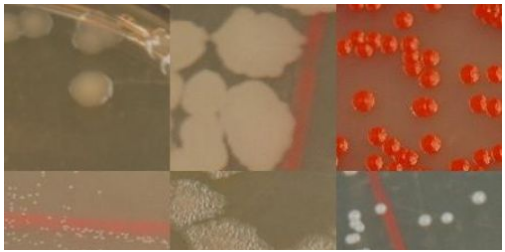
Preservation of Cultures:

- Pure cultures of bacteria are stored:
    - Freeze-dried (lyophilized)
    - Frozen at -80C

Identification

- Colony morphology





- Cellular morphology

- Microscope
    - Resolving power (resolution) = ability to distinguish between 2 closely located objects as separate, distinct entities

<u>Staining Techniques</u>

- 3 steps:
    1. Make a smear
    2. Fix dried smear permanently on the glass by heat
    3. Stain with desired dye

Simple vs Differential Staining

- Simple stain
    - Single dye normally used
    - All organisms same colour
    - Size, shape, number, arrangement, etc.
    - Most of the time not very helpful for diagnostic microbiology
- Differential stain
    - This one is more important for diagnostic microbiology because…
        - 2 or more dyes
        - Differences b/w microorganisms or parts of cells
        - Acid fast, Gram

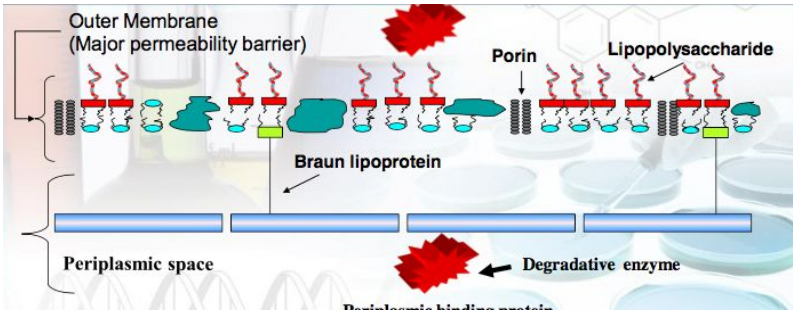
The Gram Stain

1. Flood slide with crystal violet (wash with running tap water)
2. Flood with Gram's iodine (wash with water)
3. Carefully decolorize with 95% ethanol (wash with water)
    - Most critical and also the one most affected by technical variations in timing and reagents
4. Flood with safranin (pink colour) (wash with water). Air dry, or blot with absorbent paper

Cell wall is the key!

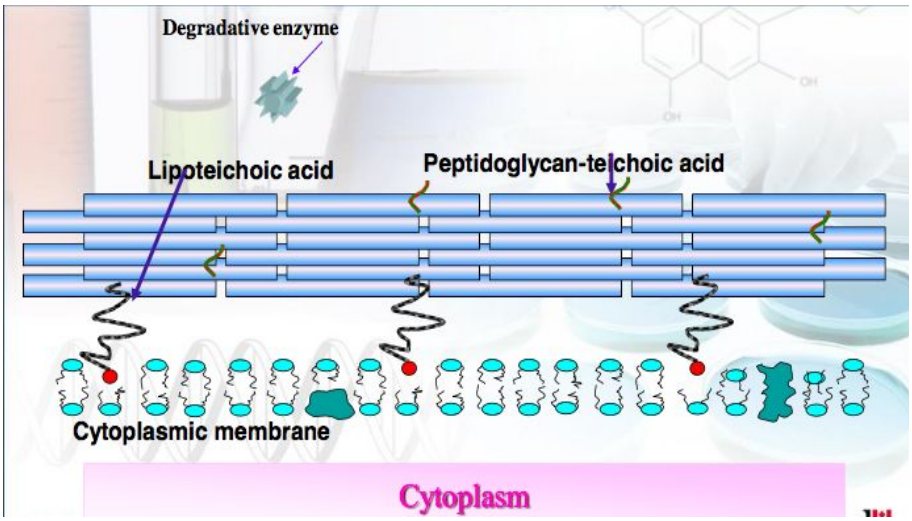
- Essential for cell growth and division
- Shape of bacteria related to peptidoglycan layer
- Gram negative usually thinner than gram positive

Gram negative cell envelope:



- Lipopolysaccharide (an endotoxin) is only found in the gram negative cell wall
- so if someone is showing signs of toxic shock, you know it can't be a gram positive bacteria

Gram positive cell envelope:



Other stains:

- Endospore
- Capsule
- Flagella

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
    - TEM (stain with heavy metals)
    - SEM (3-D image of cell surface)

Characteristics of bacteria

- Small
- Higher surface area/ volume ratio

- Higher metabolism
- Faster growth
- Replication rate (~20 mins)

Shapes and Sizes

- 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 3 planes (grapelike clusters)
    - Cubical packet of 8 cells (sarcinae)

- Chemically defined: exact composition known
- Chemically undefined: some components can't be controlled (beef extract, blood, etc.)
- If solid (vs liquid growth) -- 1.5% agar used
- 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
    - Viruses or parasites cannot multiply on their own
    - To mimic the nutrients they need, they use live cells

General Media Requirements

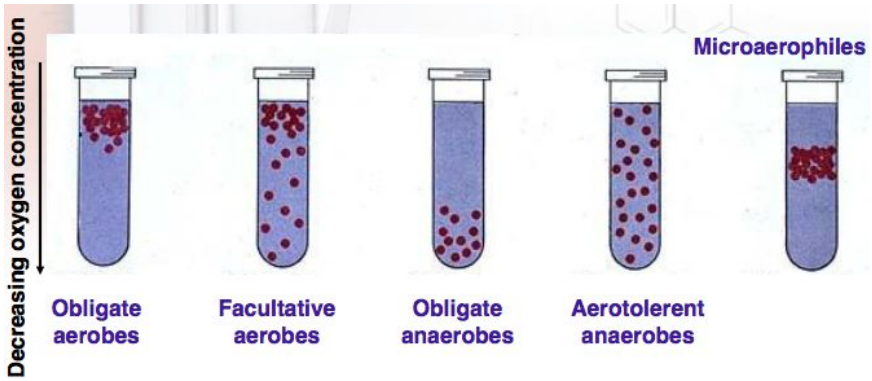
- Bacteria - requirements vary
- Yeasts - high sugar and lower pH
- Anaerobes - must remove oxygen

- Selective media: enhance growth of one bacteria species or another
- Differential media: differentiate bacteria based on their nutritional requirements and phenotypic characteristics
- Selective/differential media: very useful in clinical labs (uses both above techniques)
    - Eg. MacConkey agar
        - Has bile salts, crystal violet inhibit Gram positives (only allows gram negatives to grow)

Temperatures

- Psychrophiles
    - Grow best at temps 15-20C
- Mesophiles
    - Grow best at temperatures 25-40C
    - Most bacteria belong here
- Thermophiles
    - Grow best at temperatures 40-85C

Oxygen Requirements



pH and 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
- Osmostic pressure (hypertonic, hypotonic, isotonic)

GRAM POSITIVE COCCI

<u>Staphylococcus aureus</u>

- “Staphule” means grape in greek
- Toxins are the problem:
    - Cytotoxins - toxic to many cells: leukocytes, erythrocytes, macrophages, platelets and fibroblasts
    - Haemolysins - dermonecrotic, lethal
    - Enterotoxin (A-E, G-I)
        - Superantigens
        - important cause of food poisoning (nausea, vomiting, cramps, diarrhoeas)
    - Exfoliative toxins (ETA, ETB) - "scalded skin" syndrome in infant
    - Toxic shock syndrome toxin 1 (used to be exotoxin C and enterotoxin F)
- Enzymes
    - Coagulase (coagulation of fibrin
        - Made by almost all pathogenic staphylococci
        - Used in laboratory to differentiate from S. epidermidis, S. capitis, and S. saprophyticus

        - Beta-lactamase (penicillinase)
            - Destroys penicillin
- Many S. aureus strains are found is 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, conjuntivitis, otitis, etc.)
    - Pneumonia, osteomyelitis, septicaemia, endocarditis
    - Food poisoning, toxic shock syndrome, scalded skin syndrome
- Important cause of hospital acquired nosocomial infections from stitch abscesses, infected wounds, or generalized infections
- Preventative measures include
    - Aseptic technique in ER and OR, wound precaution
    - Education of health personnel
    - Handwashing!

<u>Staphylococcus epidermidis</u>

- Part of normal skin/mucous membrane flora
- Non-pathogenic, except in compromised patients where it can cause post-operative infections (brain, open-heart, endocarditis, shunt infections)
- Considered an opportunistic pathogen

Streptococci

- Arranged in pairs or forming chains
- “Streptos” - greek word for twisted
- Subdivided into “groups” based on
    - Haemolytic properties (alpha, beta)
    - Carbohydrate C antigen (Lancefield classification)
    - M-protein
        - Divides beta-haemolytic
        - Mostly group A

<u>Streptococcus pyogenes</u>

- Group-A, beta-hemolytic, S. pyogenes causes:
    - Acute tonsillitis (strep throat) - can lead to rheumatic heart disease
    - Impetigo, cellulitis, etc, (skin infections)
    - Fever and septicaemia
- Caused by toxins
    - Streptolysins (O and S)

        - Neutrophils and macrophages
    - Streptococcal pyrogenic exotoxins (Spes)
        - Scarlet fever rash
- Enzymes
    - Hyaluronidase (helps spreading of bacteria)
- Virtually all are penicillin G sensitive (vs S. aureus)
- Education of health personnel
- Aseptic obstetric procedures
- Early detection and treatment

Flesh-eating disease (aka Necrotizing fasciitis)

- Streptococcus pyogenes culprit
- Does not actually “eat” anything
- Toxin is responsible for damage
- Research indicates that
    - 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 (WBCs)

<u>Streptococcus agalactiae</u>

- Group B
- Found in vagina of healthy women (can cause neonatal infections)
    - Early septicaemia
        - Respiratory distress or shock at birth
        - High fatality rate (serious)
    - Delayed meningitic form
        - 1-12 weeks postpartum
        - Sequelae

Streptococcus faecalis

- Group D, aka Enterococcus
- Part of normal flora of GI-tract
- prey on compromised individuals

Viridans streptococci

- Found in oral cavity of healthy individuals
- Can cause endocarditis in individuals with damaged heart valves

Streptococcus pneumoniae

- Also known as pneumococcus
- Polysaccharide capsule has antiphagocytic properties
    - ~90 distinct capsular serotypes
- Found in nasopharynx of healthy individuals
- Can cause:
    - Lobar pneumonia
    - Meningitis
- Prevention strategies (elderly, alcoholics, crowded living, vaccination)

GRAM NEGATIVE COCCI

<u>Neisseria Meningitidis</u>

- Gram negative diplococci
- Lab isolation using chocolate agar, 5-10% CO2, 37C
    - Use selective media (ie. Thayer-Martin) when isolating from nasopharynx
- Frequently found in the nasopharynx 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 an 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 (complication of septicaemia - most severe form of septicaemia by N. meningitidis)

Meningitis

- First described in 1894 by Arthur Francis Voelcker (1861-1946)
- Then in 1901 by the British dermatologist Ernest Gordon Graham Little (1867-1950)
- It was first reported as an entity by Waterhouse in 1911, and the subject was comprehensively reviewed in 1918 by the Danish paediatrician Carl Friderichsen

- Prevention and treatment
    - 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 W135v
    - Now have meningococcus vaccine for infants at 2-5 months (serogroup C)

Neisseria gonorrhoeae

- Gram negative diplococci, 0.6-1μm in diameter
- In a clinical lab, grow on Thayer-Martin plates, in damp envt with CO_2
    - Very sensitive to drying and changes in temp
- Causative agent of STD gonorrhea
- In US, it's the second highest reported STD, after chlamydia
    - More than 350,000 cases/year reported in the US (2001)
    - # of cases is now decreasing every year
- Clinical gonorrhea
    - Men: causes acute infection of urethra
    - 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 ofloxin combined with doxycycline/azithromycin
    - Resistance to ciproflaoxacin (quinolones) emerging
    - Simultaneous treatment of partners is ESSENTIAL
    - No vaccine available