Guide for use of these notes

First of all thank you for choosing to download these notes to study from I hope you find them useful, please feel free to email me if you have any problems with the notes or if you notice any errors. I don’t promise to respond to all emails but I’ll do my best.

I organise my notes so that you should read the learning objectives on the left then proceed down the right hand side for a few learning objectives and then cross back over to the left and continue like that.

Anything in this highlighted green is a definition or explains basically something’s function.

🌟 Text highlighted in yellow or with a star is what I would deem important and key information.

Italics and bold just help to make certain terms stand out.

The notes are a bit quirky but I hope you like them and find some of the memory aides strange enough so that they stick in your head.

I provide them to you in OneNote format as that is how I created them, they can be saved as PDF but the formatting is not as nice. The one caveat with this is that these notes are freely copy able and editable. I would prefer if you didn’t copy and paste my notes into your own but used them as a reference or preferably instead embellished these already existing notes by adding to them.

Good luck with first year

Stuart Taylor
**Gram staining**

<table>
<thead>
<tr>
<th>Gram Positive</th>
<th>Gram Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptidoglycan in cell wall retains dye</td>
<td>High peptidoglycan= deep violet</td>
</tr>
</tbody>
</table>

**Three types**

- **Cocci** - round
- **Bacilli** - Rod
- **Spirilli** - Spiral

**Outnumbered 10:1 in terms of cells by bacteria in our body (intestines)**

**Gram negative**
- Escherichia coli (EPEC - diarrhoea, EHEC - dysentery and kidney failure)
- Salmonella (typhimurium - food poisoning, typhi - typhoid) Causes enteritis and typhoid fever.
- Shigella (dysentery)
- Vibrio cholerae (choleragen)
- Neisseria (meningitidis- meningitis, gonorrhoeae causing gonorrhoea)

**Gram positive**
- Staphylococcus aureus (skin diseases, endocarditis, bacteraemia, joint diseases, pneumonia)
- Streptococcus pneumoniae (pneumonia, meningitis, otitis media)
- Streptococcus pyogenes (tonsillitis, necrotizing fasciitis, bacteremia, scarlet fever)
- Listeria

**Mycobacteria**
- Mycobacterium tuberculosis (TB)
- Mycobacterium leprae (leprosy)

- Bacteria are small and unicellular
- They have no internal organelles (no chloroplasts, nucleus, ER, mitochondria)
- Have no flagella
- Some have flagella

**Learning Objectives**

- Main difference between Gram + and Gram – bacteria
- Examples of intracellular and extracellular bacteria
- Flagella and type III secretion – 2 related bacterial multi-protein machines
- Examples of manipulation of host actin cytoskeleton: bacterial entry and movement
- Mechanisms of horizontal gene transfer
- Genome diversity and evolution

**Wikipedia entry: Treat with caution**

<table>
<thead>
<tr>
<th>Number</th>
<th>Gram Positive</th>
<th>Gram Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Thick peptidoglycan layer (which is much thinner than in Gram-positive bacteria)</td>
<td>Thin peptidoglycan layer</td>
</tr>
<tr>
<td>2.</td>
<td>Outer membrane containing lipopolysaccharides (LPS, which consists of lipid A, core polysaccharide, and O antigen) outside the peptidoglycan layer</td>
<td>None exist in the outer membrane</td>
</tr>
<tr>
<td>3.</td>
<td>Pore in the outer membrane, which act like pores for particular molecules</td>
<td>Porins exist</td>
</tr>
<tr>
<td>4.</td>
<td>There is a space between the layers of peptidoglycan and the secondary cell membrane called the periplasmic space</td>
<td>No space</td>
</tr>
<tr>
<td>5.</td>
<td>The 5-layer is directly attached to the outer membrane, rather than the peptidoglycan</td>
<td>No direct attachment</td>
</tr>
<tr>
<td>6.</td>
<td>If present, flagella have four supporting rings instead of two</td>
<td>No flagella present</td>
</tr>
<tr>
<td>7.</td>
<td>No teichoic acids or lipoteichoic acids are present</td>
<td>Teichoic acid presence</td>
</tr>
<tr>
<td>8.</td>
<td>Lipoproteins are attached to the polysaccharide backbone</td>
<td>None attached</td>
</tr>
<tr>
<td>9.</td>
<td>Most of them contain Braun’s lipoprotein, which serves as a link between the outer membrane and the peptidoglycan chain by a covalent bond</td>
<td>None contain Braun’s lipoprotein</td>
</tr>
<tr>
<td>10.</td>
<td>Most do not sporulate (Coxiella burnetii, which produces spore-like structures, is a notable exception)</td>
<td>Some do sporulate</td>
</tr>
</tbody>
</table>

**Examples of intracellular and extracellular bacteria**

**Gram staining**

- **Gram positive**
  - Peptidoglycan in cell wall retains dye
  - High peptidoglycan= deep violet

- **Gram negative**
  - Outer membrane resists the dye.
  - The cells absorb counterstain making them appear pink.
Pathogenicity island: horizontally acquired DNA that contributes to virulence

**Genomic Diversity and Evolution**

- **Incredible source of genetic variation**
- Rapid generation time
- Selective pressure
- Approx 1000,000,000 years
- Evolution

**Bacterial Pathogens**

- Colonize (surface structures such as pilus, fimbriae)
- Persist (avoid, subvert, or circumvent host defences in or outside cells)
- Replicate (acquire nutrients such as iron, energy sources etc.)
- Disseminate within cells, tissues between organs and hosts (bacterial and host cell motility, through aerosols, faeces etc.)
- Cause disease (produce toxins that kill host cells, induce diarrhoea, deregulate immune responses)

**Motility and invasion requires 2 multi-protein machines**

- **Type III secretion system**
  - Flagella

**Mechanisms of horizontal gene transfer**

- **Transformation** - Uptake of double stranded DNA by uptake proteins and integrated with homologous recombination.
  - Examples: Neisseria and Streptococcus

**Horizontal Gene Transfer**

- **Transformation** - Uptake of naked DNA in cytoplasm
- **Conjugation** - Protein bridge linking bacterial cells, can transmit plasmids
- **Transduction** - Transmission by bacteriophages (bacterial viruses)

**Intracellular and extracellular pathogens**

- Extracellular: Staphylococcus, Streptococcus, Yersinia, Neisseria
- Some intracellular pathogens such as Listeria, Shigella can break out of vacuole made from plasma membrane and thus ESCAPE!!!
- Salmonella and Mycobacteria stay within the vacuoles and modify membrane so it cannot fuse with lysosomes so hydrolytic enzymes cannot kill them.
- Neisseria and salmonella are facultative intracellular - they can live inside or out of cells.

**Listeria** the gram positive bacteria is phagocytosed but manages to escape from the vacuole it is placed in. Listeria then takes advantage of the actin cytoskeleton and creates a rocket tail for itself.

- Listeria assemble an actin tail at one pole of bacterial cell to propel them more.
- Then swim randomly until reaching plasma membrane whereby they try and go to next cell.

**From Booklet**

Bacteria can be broadly divided into two groups on the basis of the Gram stain.

- Gram positive bacteria retain a violet dye in the cell wall. The stain is excluded from Gram negative bacteria by the presence of an outer lipopolysaccharide membrane.
- Most bacteria are harmless or beneficial but a few are pathogenic. Salmonella typhi causes *typhoid* Shigella causes *shigellosis*. Two species of Neisseria cause two very different diseases, meningitis and gonorrhoea. Pneumonia can be caused by Streptococcus pneumonia and tuberculosis is caused by Mycobacterium tuberculosis.

To be a pathogen, a bacterium must be able to colonize, persist, replicate and disseminate. Bacterial pathogens can be extracellular (e.g. *streptococcus*) facultative intracellular (e.g. *Salmonella and Neisseria*), or obligate intracellular (e.g. *Chlamydia*).

Salmonella is motile and invasive. Motility is due to flagella and bacteria invade host cells using a **Type III secretion system** through which they transfer virulence proteins into the host cell. These cause actin polymerisation, membrane ruffling and uptake of bacteria.

Listeria invades host cells and then breaks out of the membrane-bound vesicle. Once in the cytoplasm of host cells, they move around and spread from cell to cell by polymerising actin at one pole of the bacterial cell.

Variation in vertically transmitted DNA can occur as a result of **mutation** DNA can also be acquired by horizontal transfer.

The three basic mechanisms of horizontal gene transfer are **transformation**, **transduction**, and **conjugation**.
horizontal transfer.

The three basic mechanisms of horizontal gene transfer are **transformation**, **transduction**, and **conjugation**.

Genomes of bacterial pathogens can encode between 1000 and 5000 proteins, depending on the species. Only a small proportion of these are related to pathogenicity. These virulence genes are frequently found on **pathogenicity islands**. Distinguishing features of these elements are 10-200kb in size, are discrete genetic units flanked by direct repeats and tRNA genes. Because the doubling time of bacteria can be very short (as low as 20 mins) they can achieve vast numbers in short time-frames.


**Properties of bacterial pathogens: self test**

**Questions**

1. What distinguishes Gram-positive from Gram-negative bacteria?
2. What attributes of bacterial pathogens distinguish them from non-pathogens?
3. Give examples of pathogens that replicate mainly extracellularly.
4. Give examples of pathogens that replicate mainly intracellularly.
5. What multi-protein machine enables Salmonella and many other bacteria to move?
6. What multi-protein machine enables Salmonella to invade host cells?
7. How does Listeria move inside host cells?
8. What are ‘core’ and ‘accessory’ genes?
9. What are the main means by which bacteria exchange genetic material?
10. What is a ‘Pathogenicity Island’?

**Additional information**

Flagella made up of rods and rings.

Rings are formed first in inner membrane. Then rods go through middle. Assembly by capping proteins from the top. Flagella motor cause rotation and movement.

- Bacterial Pathogenesis: A Molecular approach (Salyers and Whitt) (2nd Ed)
- Cellular Microbiology (Cossart, Boquet, Normak, Rappuoli) (2nd Ed)

**Conjugation**

- Conjugative transfer of a self transmissible plasmid
  - Mating bridge forms between two bacteria: each bacteria only has one strand of DNA
  - Each bacteria synthesises a new complementary strand to the plasmid.

Examples: Many Gram + and -

**Transcomplementation**

- Donor
- Recipient

**Bacterial chromosome**

- Donor DNA
- Newly synthesized DNA
Learning Objectives

- List the potential sources and possible routes of infection by bacteria
- Explain the concepts of infectivity and virulence and define the term infective dose
- Give examples of bacterial pathogens transmitted by different routes and outline the ways in which they cause disease
- Explain the concepts of infectivity and virulence and define the term infective dose

Potential sources and possible routes of infection by bacteria:

<table>
<thead>
<tr>
<th>Intrinsic</th>
<th>Examples</th>
<th>Extrinsic</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal cavity, sinuses and URT</td>
<td>Staphylococcus</td>
<td>Secretions from Mouth, Nasal Cavity, URT</td>
<td>Influenza, Rhinovirus, Neisseria meningitidis and Streptococcus pneumonia</td>
</tr>
<tr>
<td>Stomach</td>
<td>Helicobacter pylori</td>
<td>Contaminated Food and Water</td>
<td>Hep A, Norovirus, Vibrio cholera, Enterotoxins</td>
</tr>
<tr>
<td>Skin</td>
<td>Staphylococcus epidermidis</td>
<td>Sexual Transmission</td>
<td>Neisseria gonorrhea, Syphilis</td>
</tr>
<tr>
<td>Intestines</td>
<td>Many gram negative E. coli, Clostridium Spp.</td>
<td>Nosocomial (health care workers)</td>
<td>Staphylococcus aureus, Clostridium difficile (Patient → Patient)</td>
</tr>
</tbody>
</table>

Pathogens cause disease.
- True: Staph aureus abscesses
- Opportunistic: Staph epidermidis giving hip infection

Infectivity: General features that aid the infection of a host
- Transmission to host
- Ability to colonise
- Replication
- Tropism: Unique niche e.g. Salmonella, Mycobacterium & Shigella are intracellular
- Immune Evasion at site of colonisation or niche.

Virulence: Features that enhance disease causation
- Toxin production
- Enzymes that degrade host molecules
- Complete immune system evasion - HIV
- Interference with host cell function

Enteropathogenic E. Coli causes imbalance in chloride transport that causes transport of chloride ions and water into bowel thus causing diarrhoea. Opening of ion channels!

The infectious dose is the number of bacteria required to initiate an infection.

Variates between bacteria:
- Shigella 10 Colony Forming Units (CFU)
- Staph aureus - 10⁴ - 10⁵

Give examples of bacterial pathogens transmitted by different routes and outline the ways in which they cause disease

- **Upper → Lower Respiratory Tract:**
  - Bacterial migration to lower respiratory tract. Pneumococcus is normal URT flora however when it migrates downwards it becomes dangerous.
  - **Lower gastrointestinal → Urogenital tract:**
    - E. Coli, Enterococci and Candida are likely to cause UTI.

Portals of Entry

- **Broken Skin**
  - Streptococcus pyogenes can infect wounds in the mouth.

- **Injury**
  - Insects
  - Pre existing skin breaches cuts, burns etc.

- **Intravenous Drug Abuse**

- **Pre existing skin breaches cuts, burns etc.**

- **Mouth droplet transmission:**
  - Tonsillitis - Streptococcus pyogenes
  - Meningococcal septicaemia - Neisseria meningitidis asymptomatically colonises nasopharynx before it invades endothelial cells.

- **Upper to lower respiratory tract:**
  - Nasal Sinuses- (Potts Puffy Tumour) Strep. pneumonia and Haemophilus influenza

- **Inoculation through skin:**
  - Staph aureus- Produce family of Leukocidins which are toxins that destroy neutrophils which causes pus

- **Faeco-oral transmission:**
  - Vibrio cholerae- Mucus penetrates cells, A + B toxins cause chloride efflux, Na and Water follow which results in profuse diarrhoea and **sudden onset** of rice water stools.
Learning Objectives

- Understand the scale of the problem
- Name the important bacterial pathogens that are multi-drug resistant
- Mechanisms of antibiotics
- Mechanisms of antibiotic resistance
- Sources of antibiotic resistance genes
- Outline reasons for the high rate of hospital acquired infections
- Describe methods to reduce emergence of new resistant strains and nosocomial infections

Understand the scale of the problem

- Antibiotic resistance is recently becoming an issue within a health care setting but also in the community.
- The arrival of a new antibiotic can be the catalyst for resistance to develop.
- This is because a new antibiotic causes selection pressure within a population of bacteria and thus they by the process of evolution they become resistant to it.
- Most major antibiotics have reported resistance and a large number of serious pathogens have multiple resistance.
- Associated with increased morbidity, mortality, length of hospital stay and cost.

Mechanisms of antibiotic resistance

1. Altered target site- Can occur when the bacteria receives an alternate gene or a gene that codes for a target modifying enzyme.
   - Examples
     - Staphylococcus aureus codes for PBP2a a different version of a membrane bound protein that stops Penicillin type antibiotics from working.
     - Enterococcus faecalis and NDM 1 are also examples.

2. Inactivation of antibiotic- Enzymatic alteration or degredation of antibiotic so it is no longer effective
   - Examples
     - Beta-lactamase (BLA) and Chloramphenical acetyl transferase (cat)
     - ESBL and NDM 1 are also examples.

3. Altered metabolism- Increasing production of a substrate can competitively inhibit an antibiotic thus lower its effects. Likewise change of a metabolic pathway can completely render the antibiotic useless.
   - Examples
     - Increased production of PABA interferes with sulfonamides inhibitory effect.

4. Decreased drug accumulation- This can be achieved through two different methods of action. Firstly the bacteria can reduce permeability to the AB or it can increase efflux of it by using ATP and a pumping mechanism.

Outline reasons for the high rate of hospital acquired infections (HAI)

- Large number of infected people receiving large doses of antibiotics creates a selection pressure for the bacteria.
- High number of ill people
- Crowded wards
- Presence of pathogens
- Broken skin- surgical wound, catheter.
- Disruption of normal flora by AB therapy potentially selects for pathogens.
- Indwelling devices- intubation

Describe methods to reduce emergence of new resistant strains and nosocomial infections

Nosocomial infections- Infections that are a result of treatment in a hospital or a healthcare device unit

- Better prescribing practises- not for viral infections in GP.
- Modify existing strains to make them more effective, E.g. prevent cleavage of beta-lactams.
- Combination of antibiotic and inhibitor to make it more effective.
- Infection control measures- health care worker cleanliness.
- Use of narrow spectrum antibiotics rather than broad spectrum.

Mechanisms of antibiotics

- Penicillin Binding Proteins (PBPs)
  - On the surface of the cell wall. Bacteria such as penicillin and methicillin bind to Penicillin Binding Proteins (PBPs) on the surface of the bacteria and inhibit synthesis of peptidoligycan. Apart from PBP2a of MRSA.
  - Penicillin interferes with the synthesis of peptidoglycan. Apart from PBP2a of MRSA.
- Protein synthesis: Bacteroiatic- Erythromycin, chloramphenical & tetracycline.
- Blocks tRNA binding to binding to certain subunits on the ribosome.
- DNA Replication: Quinolones are bactericidal and interfere with enzymes in DNA replication.

<table>
<thead>
<tr>
<th>Resistance Mechanism</th>
<th>Antibiotic affected</th>
<th>Acquired by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillinases</td>
<td>Penicillin</td>
<td>Plasmid transformation</td>
</tr>
<tr>
<td>Target site modification</td>
<td>Penicillin</td>
<td>Point mutation</td>
</tr>
<tr>
<td>Efflux pump (Decreased drug accumulation)</td>
<td>Tetracycline</td>
<td>Plasmid-conjugation</td>
</tr>
<tr>
<td>Target site modification</td>
<td>Quinolone</td>
<td>Point mutation</td>
</tr>
</tbody>
</table>
Qu. 3: In the transfer of antibiotic resistance, which of the following can move small DNA elements into bacterial chromosomes or onto plasmids?

- Plasmids
- Transposons
- Integrons
- Organons
- X Bacteriaphage

**Best Option:** Transposons

Transposons are sequences of DNA that can move around to different parts of a cell’s genome, carrying with them resistance genes. Plasmids are circular, extrachromosomal, autonomously-replicating DNA and integrons are gene cassettes which occur in clusters. Organons have nothing to do with this process. Bacteriaphages are bacterial viruses, which can move DNA from one bacterium to another and integrate into the chromosome.
Learning Objectives

- Describe the nature of viruses: their small size, dependence on a host and their structural and genetic diversity
- Define the following terms as used in the description and classification of viruses: DNA Virus, RNA virus, capsid, enveloped, non-enveloped.
- Describe a genetic virus replication cycle
- Describe how viruses are detected, cultivated and manipulated.

Koch’s Postulates

1. The microorganism must be found in large numbers in all diseased animals, but not in healthy ones.
2. The organism must be isolated from a diseased animal and grown outside the body in a pure culture.
3. When the isolated microorganism is injected into other healthy animals, it must produce the same disease.
4. The suspected microorganism must be recovered from the experimental hosts, isolated, compared to the first microorganism, and found to be identical.

Define the following terms as used in the description and classification of viruses: DNA Virus, RNA virus, capsid, enveloped, non-enveloped.

DNA virus
RNA virus
Capsid - A protein coat encloses the genome and core proteins, consisting of capsomeres (capsid subunits)
Envelope - Lipid bilayer membrane surrounding the capsid of some viruses (enveloped viruses) the envelope carries glycoproteins, which forms projections or spikes. This lipid layer is acquired as the virus buds through the host cell’s cytoplasmic membrane.
Non-enveloped

Prions are infectious proteins, they don’t have genomes. Mad Cow Disease. CJD.

Describe a genetic virus replication cycle

Attachment of the viral capsid (naked viruses) or of envelope components (enveloped viruses) to cell surface molecules (receptors); this involves specific interaction between viral glycoproteins (e.g. the haemagglutination of influenza) and host cell surface components (e.g. N-acetylneuraminic acid for influenza virus). Many viruses have highly specific receptors, which limits the range of cell types that can be infected.

1. Penetration of the virus into the host cell (often by receptor mediated endocytosis)
2. Uncoating follows, which involves the enzymatic removal of viral protein coat and liberation of nucleic acid and attach core proteins.
3. RNA or DNA
   a. Single or double stranded
   b. Positive sense or negative sense
   c. Production of virus specific mRNA, in order to direct the host cell ribosome to produce viral proteins (core, capsid). The mechanisms for virus-specific mRNA production depends on the viral genome type.
4. Morphogenesis and maturation occur with assembly of components (nucleic acid and proteins) to form sub-viral particles (virions, empty particles)
5. Release of virus by bursting of infected cells or by budding through the plasma membrane. Lysis results in cell death but budding doesn’t necessarily kill cells, allowing viral particles to be shed for extended periods.

Describe how viruses are detected, cultivated and manipulated.

- Viruses can be cultivated in egg cells after inoculation, into a potentially wide variety of regions such as the yolk sac and amnion.
Cytopathic effect (CPE) - some viruses can be recognised by their effect on the appearance of the inoculated cell layer, e.g. necrosis, lysis, rounding, ballooning or the formation of multinucleated giant cells (syncytia).

a. Haemadsorption: viruses expressing haemagglutins on the cell's surface may be recognised by adsorption of human group O red blood cells. On lower power microscopy, clumpy blood cells can be seen attached to virally infected cells. If no virus is present, then red blood cells remain dispersed in liquid media. This technique is useful if no CPE is observed (e.g.) cells infected with influenza virus do not produce a CPE but do haemadsorb.

b. Immunofluorescence - the appearance of virus-encoding proteins on the surface, nucleus or cytoplasm of infected cells may be detected by immunofluorescence techniques (IFT). IFT utilises antibodies which have been chemically labelled with a fluorescent dye, that are specific to viral proteins. Examination of cell cultures with a microscope (using an ultraviolet light source enables visualisation of fluorescing cells to show infection has taken place.

c. Virus replication in cell cultures may be detected using a bench top microscope by observing:

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c. Virus replication in cell cultures may be detected using a bench top microscope by observing:

- Cell culture techniques have been largely replaced by PCR for DNA viruses and reverse transcriptase PCR for RNA viruses.

Diagnosis

- Detecting viral genome PCR
- Detecting viral antigen IFA, ELISA
- Detecting virus particles EM, HA
- Detecting virus cytopathic effect in cultured cells (Virus isolation)
- Detecting antibodies to virus (serology)
Fungal Questions

1. List the major differences between fungi and bacteria
2. What are the three major types of fungal disease?
3. Define mycotoxicoses
4. A patient has Tinea pedis – where is the infection?
5. Chromoblastomycosis causes superficial infection?
6. What are the three major systemic mycoses?
7. List the major risk factors for each type of systemic mycosis
8. What are the major targets of antifungals? Classes?

Question 1:
1. Fungi are dimorphic hypophae or yeasts
2. Fungi lack chloroplasts
3. Fungi have membrane bound organelles because they are eukaryotes.
4. Their cell membrane contains ergosterol
5. Don’t divide by binary fission they divide by “budding out”
6. Cell walls contain glucans and chitins.

Learning Objectives

- Outline the main differences between fungi and bacteria
- Summarise briefly the ecology and epidemiology of infectious fungi
- List the major groups of pathogenic fungi and their growth forms
- Define superficial mycoses and deep mycoses with examples
- Describe briefly the main classes of antifungal agents

Questions

Question 1:
1. Fungi are dimorphic hypophae or yeasts
2. Fungi lack chloroplasts
3. Fungi have membrane bound organelles because they are eukaryotes.
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6. Cell walls contain glucans and chitins.

Question 2:

1. Superficial
2. Cutaneous
3. Systemic

Question 3:

- **Mycotoxoses**: These are secondary metabolites of yeasts that have toxic effects in humans or animals.

Question 4:

- In their foot/feet.

Question 5:

- Incorrect chromoblastomycosis is a subcutaneous infection.

Question 6:

Question 7:

1. **Cryptococcus**
   - Most common is C. neoformans which is an encapsulated yeast
   - Most lethal infection in aids (meningitis)
   - Survives in environment (pigeons and eucalyptus)
   - Very high mortality (especially in sub-Saharan Africa)

2. **Candida albicans**
   - Grow as yeast and filamentous cells.
   - Opportunistic commensal fungi that needs immunocompromisation such as gut surgery, chemo and catheters. Able to cause superficial, mucosal and systemic infection.
   - Infection has four stages:
     1. Colonization
     2. Superficial infection
     3. Deep seated infection
     4. Disseminated infection

3. **Aspergillus**
   - A. fumigatus= 90% disease
   - Multicellular filamentous fungi (not yeast!)
   - Major risk factors:
     - Neutropenia
     - Transplants (lung and HSCT)
     - Leukaemia
     - AIDS
   - Spores ingested, germination, hyphal elongation and branching which generates a mass of hyphae which again causes spore release etc it’s a cycle.
   - Range of disease dependent on host response:
     - Aspergillosis in cavitory lung disease
     - Chronic necrotising aspergillosis in chronic lung disease
     - Invasive pulmonary aspergillosis in immunocompromised patients.
     - ABPA in asthma
     - Nothing in a normal host!

**Questions**

1. **Superficial Mycoses**
   - Skin or nail shafts -> no living tissue so no cellular response
   - Examples:
     1. Black and white piedra
     2. Pityriasis versicolor
     3. Tinea nigra

2. **Cutaneous Mycoses**
   - Eat keratin
   - Dermatophytes/keratinophilic fungi
   - Host responses to bi-products causes inflammation.
   - Examples:
     1. Tinea capitis= head, pedis= foot, corporis=body, cruris=groin, ungulium=nail.

3. **Subcutaneous Mycoses**
   - May be caused by traumatic implantation causing chronic skin and subcutaneous infection.
   - Examples:
     1. Sporotrichosis
     2. Chromoblastomycoses
     3. Mycetoma

Describe briefly the main classes of antifungal agents

Question 8:

- **Cell membranes**
  - **Azoles**
    - Ketoconazole
    - Itraconazole
    - Fluconazole
    - Voriconazole

- **Polymers e.g. Amphotericin B and Nystatin**
  - RNA/DNA synthesis:
    - Fluconazole: pyrimidine analogue inhibits DNA and RNA synthesis
  - Cell wall synthesis:
    - Echinocandins: inhibits 1,3 glucan synthase. Caspofungin.

**Questions**

9. The following statement is incorrect with regards to cell membrane active antifungals

- Amphotericin B is a polyeptide antibiotic and attacks the cell membrane
- They inhibit synthesis of ergosterol, which is the main sterol (as opposed to cholesterol) in the fungal membrane
- They act on the fungal cytochrome P450 enzymes
- Ketoconazole and Itraconazole are examples of azole antifungals
- Nystatin and amphotericin B work by stimulating ergosterol

**Mark = 3 (conf=3)**

Best Option: Nystatin and amphotericin B work by stimulating ergosterol

Nystatin and amphotericin B are both polyene antibiotics aimed at INHIBITING ergosterol found in the fungal membrane.
Best Option: Nystatin and amphotericin B work by stimulating ergosterol
Nystatin and amphotericin B are both polyene antibiotics aimed at INHIBITING ergosterol found in the fungal membrane

Glucan
X Mannan
Chitin
Endolase
Protease

X Mark = -6 (conf=3)

Best Option: Chitin
Chitin cannot be detected by the Ig-detected assay. The presence of any of the other proteins can help detect the pathogen that is infecting a host.
MICROBIOLOGY 6
Patterns of viral infection
Professor Wendy Barclay

Describe different routes of infection by viruses: define the term tropism and understand what defines the tropism of a virus

- Viruses can enter the body
  1. Through the epithelial layers; respiratory tract, GI tract, genital tract
  2. Directly into the blood through a bite or needle
  3. Through the skin, often following abrasion

From the site of entry the virus may travel, often in the blood (primary viraemia), to another organ where amplification via replication takes place. There may be secondary viraemia to the main organ site for replication.

The tropism of the virus is the place where it replicates.

Tropism may be determined by the expression of the host cell receptor. HIV enters cells through the CD4 molecule found on T cells.

Tropism may also be limited by the ability of the virus to replicate inside a particular cell type due to abundance or paucity of essential intracellular host cell components. Polioviruses with mutations in their 5' noncoding regions cannot utilize neuronal host cell factors to translate their mRNAs.

Tropism may also depend on extracellular factors required for activation of virus infectivity. Influenza virus HA protein requires to be cleaved by a host encoded protease expressed in respiratory secretions.

Give examples of different viruses associated with infectious disease in humans and describe their replication cycles and the way in which they cause disease

HIV (human immunodeficiency virus) is a lentivirus and a member of the retrovirus family. HIV infects and destroys helper T cells of the immune system causing a marked reduction in their numbers. Loss of CD4 cells leads to generalized failure of the immune system and susceptibility to life threatening opportunistic infections.

- gp120 – an HIV glycoprotein having a molecular weight of 120 that protrudes from the outer surface of the virion. This glycoprotein binds to a CD4 receptor on a T cell to facilitate entry of viral nucleic acid and proteins into the cell.
- CD4 – a large glycoprotein that is found on the surface of helper T cells, regulatory T cells, monocytes, and dendritic cells. Its natural function is as a co-receptor that assists the T cell receptor (TCR) to activate its T cell following an interaction with an antigen presenting cell. CD4 is a primary receptor used by HIV to gain entry into host T cells.
- Co-receptor (CCR5 or CXCR4) – protein molecules on the surface of lymphocytes, or monocytes that bind to the gp120 protein of HIV and facilitate, usually with CD4, entry of viral nucleic acid and proteins into the cell.

Fusion of virus and cell membranes – a merging of cell and virus membranes that permits HIV proteins and nucleic acids to enter the host cell.

Preintegration complex (PIC) – It is composed of viral RNA and proteins (nucleocapsid, p6, Vpr, integrase, and matrix) as well as some host proteins. It functions to reverse transcribe genomic RNA into double stranded DNA prior to integration into the host genomic DNA.

Reverse transcriptase – an enzyme found in HIV that creates double stranded DNA using viral RNA as a template and host tRNA as primers.

Integrase – An enzyme found in retroviruses including HIV that permits the viral DNA to be integrated into the DNA of the infected cell.

Protease – an enzyme that hydrolyzes or cuts proteins and is important in the final steps of HIV maturation.

Steps in the HIV Replication Cycle

1. Fusion of the HIV cell to the host cell surface.
2. HIV RNA, reverse transcriptase, integrase, and other viral proteins enter the host cell.
3. Viral DNA is formed by reverse transcription.
4. Viral DNA is transported across the nucleus and integrates into the host DNA.

Outline, with named examples, the different modes of transmission of viruses

Transmission of a virus from one host to another usually requires it to be shed into the environment.

- Rarely viruses are transmitted through the germline, acquired through cannibalism (prion diseases like Kuru) or through nosocomial blood contamination (HIV or hepatitis B or C).

Respiratory transmitted viruses are carried in aerosols (influenza, rhinovirus). Viruses may be shed into the oral cavity and transmitted in saliva (human cytomegalovirus, EBV, mumps).

Enteric viruses are transmitted through the fecal oral route (poliovirus, norovirus, hepatitis A virus)

Viraemic viruses are transmitted through blood (dengue virus when bitten by an arthropod, Ebola virus). Virus can be present in urine of animals (hantaviruses in rodents) but urine is rarely a source of human to human transmission.

Viruses in skin can be transmitted by direct skin contact, poxvirus, papillomavirus.

Describe different outcomes of infection by viruses: acute infection, persistent infection, latent re-activating infection, slow infection, oncogenesis

To be a human pathogen, a virus will need to have strategies to counteract host defenses.

The capacity of the virus to cause disease (pathogenesis) will depend on

1. the effects of its replication
2. the strength of the host’s defense system
3. the ability of the virus to spread in and amongst its hosts

Acute infection is the typical expected outcome for influenza. Rapid production of infectious virus, rapid resolution and elimination of virus by host immune system.

The outcome is determined by intrinsic and innate immunity. Acquired immunity stimulated after several days mediates final clearance from the host. Memory provides defense against subsequent exposure.

Acute infections frequently cause epidemics. Transmission occurs before symptoms. Inapparent infections (asymptomatic) are common.

Persistent infections also have to overcome innate defense at the start of infection. They are not cleared by the adaptive immune response. They may be chronic or lifelong (latent, slow).

75-85% people infected by Hepatitis C virus will not clear the virus with their CTL response. This may be because the virus rapidly mutates to escape the response by changing its T cell epitopes. Of these chronically infected people, 1-5% will develop hepatocellular carcinoma. Since more than 170 million people are infected, this accounts for up to 3 million hep carcinoma cases. Chronically infected hepatocytes are destroyed by the immune system leading to fibrous scars (cirrhosis).

The classic example of a latent virus infection is herpes simplex virus. The virus first replicates in mucosal or epidermal cells. Peripheral ganglia become infected and produce a large burst of virus that disappears after 1-2 weeks. The virus establishes a latent infection in terminally differentiated non-dividing neurons of the peripheral nervous system. Since neurons do not replicate their DNA nor divide, the HSV genome survives inside these host cells. The only evidence of the virus is the expression of RNAs known as latency associated transcripts (LATs). By this time the infected host is ‘immune’, they have antibodies to their latent virus. Some people reactivate their virus every 2-3 weeks, others experience few or no reactivation events. Stress signals can trigger reactivation. Reactivation can also be by drugs like glucocorticoids that stimulate transcription but suppress immune responses. Transient production of virions allows spread of the virus across innervated mucosal surfaces to a new host. Then the infected host’s immune response curtails virus production.
5. New viral RNA is used as genomic RNA and to make viral proteins.
6. New viral RNA and proteins move to cell surface and a new, immature, HIV virus forms.
7. The virus matures by protease releasing individual HIV proteins.
Learning Objectives

- Describe the difference between prophylactic and therapeutic approaches to virus control
  - Prophylaxis is preventing disease before the aetiological agent is acquired, by vaccination or giving drug before infection.
  - Therapy is treating the disease after the host has been infected.

- Understand the difference between a live attenuated vaccine and an inactivated or subunit vaccine

- Give examples of viral infections for which vaccinations can be a successful strategy

- Describe the eradication of smallpox and similar efforts to control other viral diseases

- Explain why it is difficult to develop drugs which selectively act against viral infections

- Give examples of classes of drugs which have been successfully used on antiviral therapy

- Describe the strategies underlying the search for novel antiviral agents

Different types of vaccine

- Different types of vaccines are attenuation: live viral vaccine grown in another organism which makes it less pathogenic in humans but still will illicit immune response.

- Inactivated fractionation: non recombinant purified subunit vaccine

Different types of viral vaccines

- Pros and cons of live vs inactivated vaccines

Live vaccine
- Rapid broad, long lived immunity
- Dose sparing
- Cellular immunity

Inactivated vaccine
- Safe
- Can be made from wild type virus

Pros
- M2 is the influenza ion channel.
- M2 is the influenza ion channel.

Cons
- Only activated inside a virus infected cells.
- Higher affinity for viral DNA polymerase than for host cell polymerase.
- Chain termination due to lack of OH group.
- Resistance is rare but maps to thymidine kinase.
- Resistance is rare but maps to thymidine kinase.

Nucleoside/tide analogues

- Azidothymidine
- Adenosine arabinose
- Dideoxycytidine
- Acyclovir

Acyclovir

- Only activated inside a virus infected cells.
- Higher affinity for viral DNA polymerase than for host cell polymerase.
- Chain termination due to lack of OH group.
- Resistance is rare but maps to thymidine kinase.

- M2 is the influenza ion channel.

Adamantanes

- Inhibits M2 ion channel which is essential for influenza replication, in particular its ability to reveal its genome.
- Cyclic amines with bulky, cage-like structures.
- By-products of petroleum refinement.
- Active against influenza A virus only.
- Side effects on CNS - causes dizziness and potential mutations in the virus can cause it to be ineffective.
- H3N2 is often resistant.

Live attenuated - Measles, mumps, rubella, influenza, polio
Purified subunit vaccine - Influenza
Cloned Subunit - Hepatitis B, Human papillomavirus

Stuart's Microbiology Page 14
Pros and cons of live vs inactivated vaccines

**Live vaccine**
- Rapid broad, long lived immunity
- Dose sparing
- Cellular immunity

**BUT**
- Requires attenuation
- May revert

**Inactivated vaccine**
- Safe
- Can be made from wild type virus

**BUT**
- Frequent boosting required
- High doses needed

**Influenza**
- Inactivated virus or HA subunit
- Updated regularly
- DOES NOT give the recipient the flu!!!

**Poliovirus**
- Salk inactivated vaccine
- Sabin live attenuated vaccine
- 1 in 7 million vaccinations associated with poliomyelitis
- Persisting in immunosuppressed individuals

**LAIV is cold adapted**
- FluMist delivered intranasally
- Updated regularly

**DOES NOT give the recipient the flu!!!**

**LAIV** is cold adapted

**FluMist** delivered intranasally

**Pasted from** https://www.ucl.ac.uk/lapt/laptlite/sys/run.htm?icl08_microbiol?f=clear?i=icl1?k=1?u=_st1511?i=Imperial

**Recombinant Vaccine**

Isolate pathogenic virus

**Clone genome**

**Receptor-binding protein gene**

**Viral genome**

**Virion protein genes**

**Capsid protein genes**

**Isolate viral genes**

The resulting virus is viable and immunogenic but not virulent. It may be used as a vaccine.

**Neuraminidase inhibitors**
- **Neuraminidase** is an enzyme that viruses produce to degrade cell surface molecules.
- Binds well to neuraminidase receptors which means virus particles stay attached to cell for immune response clear up.

1. Sialic acid
2. Oseltamivir- tamiflu
3. Zanamir- Relenza

**How do the HIV antiretrovirals work?**

- **NRTI**
  - Zidovudine, Stavudine
  - Looks like an Nucleotide but doesn’t polymerise – stops chain growth

- **NNRTI**
  - Efavirenz
  - Viramune

- **Protease Inhibitors**
  - Atazanavir (generally taken with Ritonavir)

- **Integrase inhibitors**
  - Raltegravir
  - Allosteric inhibitor

- **Entry inhibitors**
  - Enfuvirtide, CCR5, CXCR4 inhibitors
    - giv-prodrug to stop fusion

- **Reverse Transcriptase**
  - Generating DNA from RNA
  - Binds HIV gp41 preventing membrane fusion.

- **Protease inhibitors**
  - Atazanavir (generally taken with Ritonavir)

- **Nucleotide analogues**
  - Zidovudine, Stavudine
  - You need voodoo to be an HIV NUCLEOTIDE ANALOGUE

- **Allosteric inhibitor**
  - Viramune

**Describe the strategies underlying the search for novel antiviral agents**

- Create an interferon like drug which affects availability of nucleoside precursors.
- Screen already existing drugs/compounds to see if any of them have inhibitor activity.
- Fludase a bacterial sialidase that removes the receptors from target cells.

**Qu. 3:** The following statement about the Herpes simplex virus is incorrect

**HSV-1 gives genital herpes, HSV-2 gives cold sores**

Local replication is by means of entry into neurons and retrograde axonal transport to local ganglion.

In the latent stage of infection, genome becomes circular, remains in nucleus, not integrated into host DNA.

Only one area of the genome is transcribed.

**Qu. 4:** Explain why it is difficult to develop drugs which selectively act against viral infections

- Viruses operate within host cells which means that the drugs have to be highly selective for viral enzymes, proteins etc. or they will damage the host cell.
- They also take over host cell machinery which complicates the problem further as you do not want to destroy the cell.

**Qu. 5:** The following statement about the Herpes simplex virus is incorrect

HSV-1 gives genital herpes, HSV-2 gives cold sores

Local replication is by means of entry into neurons and retrograde axonal transport to local ganglion.

In the latent stage of infection, genome becomes circular, remains in nucleus, not integrated into host DNA.

Only one area of the genome is transcribed.
Learning Objectives

- Define the terms zoonosis and host range
- Define the terms zoonosis and host range
- Describe how viruses emerge and re-emerge; using named examples including influenza virus antigenic shift and drift, HIV, West Nile Virus, SARS and noroviruses
- Understand the principles of the evolution of drug resistant variants of viruses
- Understand the principles of the evolution of drug resistant variants of viruses

Define the terms zoonosis and host range

- Most viruses that infect animals do not infect humans because there is a host range barrier.
- Zoonosis: Occasionally an animal virus infects a person.
- A small outbreak may occur often with deadly consequences e.g. Ebola, Nipah.
- Only if the animal virus mutates to spread in humans will a pandemic emerge.

Describe how viruses emerge and re-emerge; using named examples including influenza virus antigenic shift and drift, HIV, West Nile Virus, SARS and noroviruses

- Evolutionary pressure drives influenza antigenic change.
  - Antigenic shift: This is the formation of a new viral subtype by the fusion of two or more viral strains which contain a mixture of both viral antigens.
  - Antigenic drift: This is the natural process of antigenic mutation which is responsible for seasonal viral strains.
- Influenza: The H stands for Haemagglutinin and the N stands for Neuraminidase which are types of cell surface molecule. Depending on which type they have it means that can infect different types of animals.
- Antigenic shift: Reassortment can occur when two viruses both infect a host cell and their genomes combine to form a new novel virus.

- Influenza: Originated in monkeys such as the Chimpanzee and Sooty mangabeys
- SARS-CoV: Originated in Bats and Civets.
- Emerged in USA in 2002 deaths rose to about 300 in end of 2002 and declined by 2008.
- Culex tarsalis is the vector for West Nile Virus
- Horses and humans are dead end hosts.

What viruses should I know about?

- Viruses have appeared due to a change in man's behaviour and in some cases their interventions. Examples include Myxoma virus released for rabbit control in Australia and genetic manipulation creates a transmissible H5N1 influenza virus.
- New viruses can be those that are only recently detected or discovered, 'Non A non B' hepatitis caused by hepatitis C virus.
- Human papillomaviruses 16 and 18 as cause of cervical cancer.
- HHV8 as cause of Kaposi's Sarcoma noticed during AIDS pandemic.

Influenza

- An enveloped particle, ss negative sense RNA genome in 8 segments.
- Replication cycle:
  a) Sialic acid receptor bound by HA
  b) Endocytosis and fusion of HA at low pH
  c) M2 ion channel a drug target amantadine
  d) Replication in the nucleus
  e) Assembly and budding from apical plasma membrane
  f) Release by NA (a better drug target, Relenza or tamiflu)

- Transmission; airborne and fomites.
- Acute infection. Antigenic drift.
- Pandemic potential by zoonosis antigenic shift.

Viral Vaccines
### Viral Vaccines

- Live attenuated: Measles, mumps, rubella, yellow fever, influenza
- Inactivated: Polio, Japanese encephalitis, rabies, hepatitis A
- Purified subunit: Influenza
- Cloned subunit: Hepatitis B S Ag, Cytomegaly virus, papillomavirus

<table>
<thead>
<tr>
<th>Live attenuated</th>
<th>Inactivated virus</th>
<th>Purified subunit</th>
<th>Cloned subunit</th>
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<tr>
<td>Adenovirus1</td>
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<td>Influenza</td>
<td>Hepatitis B</td>
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<td>Japanese encephalitis</td>
<td>Human papillomavirus</td>
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<td>Mumps</td>
<td>Rubies</td>
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<td>Polio</td>
<td>Tick-borne encephalitis</td>
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<td>Rottavirus</td>
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<td>Rubella</td>
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<tr>
<td>Smallpox</td>
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<td>Variola</td>
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<tr>
<td>Yellow fever</td>
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</table>

#### Antiviral drugs:
- Acyclovir: specificity and mode of action.
- HAART for HIV
- Neks for influenza neuraminidase action inhibitor

#### Emerging viruses:
- Dengue fever, West Nile arboviruses
- Hepatitis C virus recently discovered, blood borne.
- Nipah, hendra, bats and pigs.

#### HAART
- **Entry inhibitors** (or fusion inhibitors) interfere with binding, fusion and entry of HIV-1 to the host cell by blocking one of several targets. **Maraviroc** and **enfuvirtide** are the two currently available agents in this class.
- **CCRS receptor antagonists** are the first antiretroviral drugs which do not target the virus directly. Instead, they bind to the CCRS receptor on the surface of the T-Cell and block viral attachment to the cell. Most strains of HIV attack T-Cells using the CCRS receptor. If HIV cannot attach to the cell, it cannot gain entry to replicate.
- **Nucleoside reverse transcriptase inhibitors (NRTI)** and **nucleotide reverse transcriptase inhibitors (NtRTI)** are nucleoside and nucleotide analogues which inhibit reverse transcription by being incorporated into the newly synthesized viral DNA strand as faulty nucleotides; they both act as **competitive substrate inhibitors**.
- **Non-nucleoside reverse transcriptase inhibitors (NNRTI)** inhibit reverse transcriptase by binding to an **allosteric site** of the enzyme; NNRTIs act as **non-competitive inhibitors** of reverse transcriptase.
- **Protease inhibitors (PIs)** target viral assembly by inhibiting the activity of protease, an enzyme used by HIV to cleave nascent proteins for the final assembly of new virions.
- **Integrase inhibitors** inhibit the enzyme **integrase**, which is responsible for integration of viral DNA into the DNA of the infected cell. There are several integrase inhibitors currently under clinical trial, and **raltegravir** became the first to receive FDA approval in October 2007.
- **Maturation inhibitors** inhibit the last step in gag processing in which the viral capsid polyprotein is cleaved, thereby blocking the conversion of the polyprotein into the mature capsid protein (p24). Because these viral particles have a defective core, the virions released consist mainly of non-infectious particles.
  - **Alpha interferon** is a currently available agent in this class.  
  - **Virecon**

#### Binding
- Endocytosis
- Acidification
  - **Exocytosis**
  - Release of RNA

**Mark = 3 (conf=3)**

**Best Option:** Exocytosis

Exocytosis is not part of the influenza virus entry pathway. Instead FUSION occurs after acidification and prior to release of RNA into cytosol of host cell. Fusion occurs between the viral protein coat (HA) and intracellular vesicle.

**Qu. 11:** The following is NOT a key step of influenza virus entry into a host cell

- Transmission; airborne and fomites.
- Acute infection. Antigenic drift.
- Pandemic potential by zoonosis antigenic shift.
- Control by vaccination with inactivated vaccine to elderly of live attenuated vaccine to children.

#### HIV

- An enveloped particle with RNA genome.
- **Replication cycle:**
  - a) GP120 binds to CD4 and either CCR5 or CXCR4 on macrophage or t cells.
  - b) Entry by fusion at cell membrane
  - c) Reverse transcriptase, integration targets for AZT (3'-azidothymidine)
  - d) Protease processing for maturation of particle [Sequenovir target]
- Highly transmissible; sexual contact; blood transfusion.
- Highly antigenically variable, no vaccine available.
- HAART. (Highly Active Anti-Retroviral Therapy uses combination of anti-retrovirals)
- Origin in simian species.

#### Persistent infections:
- Papillomavirus; warts and cervical cancer in skin with low immune surveillance.
- Herpes simplex virus; cold sores; latent in terminally differentiated neurons.
- Human cytomegalovirus; control of immune system by targeting MHC.

#### Other viruses:
- Measles- Koplits spots in the mouth before skin rash; aerosol spread before visible symptoms.
- Poliovirus: accidental poliomyelitis, incidence increased after sanitation.
- Varicella Zoster Virus causes chickenpox, disseminates from the point of entry by viraemia to skin to cause rash and also being a herpes virus lays latent in neurones until reactivation causing shingles.

**Quoted:** The following is NOT a key step of influenza virus entry into a host cell

- a) Exocytosis
- b) Release of RNA
- c) Binding
- d) Endocytosis
- e) Acidification

**Best Option:** Exocytosis

Exocytosis is not part of the influenza virus entry pathway. Instead FUSION occurs after acidification and prior to release of RNA into cytosol of host cell. Fusion occurs between the viral protein coat (HA) and intracellular vesicle.
Learning Objectives

- List the major non-immune host mechanisms
- Explain the difference between active and passive immunization
- Give examples of the different types of vaccine presently available and how they are used.
- Explain how an adaptive immune response is involved in defence against bacterial pathogens
- List the components of the innate immune system and major antimicrobial mechanisms
- Describe how infectious agents avoid host defences

Non-Immune Mechanisms of Host Defence
- The skin provides a natural physical barrier to bacterial infection
- Low pH and antibacterial secretions make it a difficult surface to colonise
- We can strengthen this significantly by routine application of soap and water
- Mucosal surfaces are protected by mucous and ciliary clearance

Competition with Commensal Organisms
- Bacteria make up 60% of the earth's biomass
- The vast majority of bacteria are harmless and often helpful
- "Commensal" bacteria make up around 1% of our body mass
- "Commensal" = "eating at the same table as another"

List the components of the innate immune system and major antimicrobial mechanisms

Innate Immune Response
- Bacteria that penetrate skin and mucosal barriers are met by the innate immune response
- The complement system punches holes into bacteria
- Neutrophils and macrophages phagocytose bacteria and expose them to toxic radicals and degradative enzymes
- The immune response should be directed to the relevant site within the host
- The immune response should be functional. For example, toxin neutralizing antibodies should be able to bind to toxin and neutralize its activity or if bacterial toxins are required antibodies should bind both the bacterium and complement
- It is safe and does not cause adverse reactions
- It is inexpensive to manufacture and distribute
- It is stable
- It is easy to administer
- Should be simple for both manufacturer and regulatory authorities to control

List the major non-immune host mechanisms
- Phagocytosis by neutrophils, dendritic cells and macrophages
- Complement mediated lysis of bacterial cell membranes
- Restriction of entry to pathogens: waterproof epidermis, cilia in respiratory tract

Explain the difference between active and passive immunization
- Active immunization stimulates the host immune system whereas passive immunization does not
- Active immunity is elicited in the host in response to an antigen. Passive immunity is the acquisition of protection from another immune individual through transfer of antibody or activated T-cells. The purpose of a vaccine is to induce active immunity.

Give examples of the different types of vaccine presently available and how they are used.

Vaccine safety and efficacy are assessed in clinical trials of which there are 3 phases pre-release.

Phase 1:
- Entails the close clinical monitoring of vaccines. Data collected might include information in the following: local and systemic reactions, fever, diarrhoea and vomiting and headache etc.

Phase 2:
- Provide an opportunity to investigate the effect of different dose regimes and formulations.

Phase 3:
- Designed to investigate directly the ability of a vaccine to offer protection against disease. Efficacy is the measure of a vaccine to offer protection. Its assessment is strictly controlled (double blinded, placebo etc) and is required for licensing.
- Vaccines are made up of the antigens, adjuvant and excipients. Adjuvant is to enhance and modulate the immune response. Whereas exipients are buffers, salts, saccharides, proteins etc that maintain pH and osmolality of the vaccine.
- Live attenuated organisms. These contain mutations that affect the ability of the organism to thrive and/or cause disease in the host. Historically, the mutants were isolated by chemical mutagenesis or multiple passage of the organism; more recently attenuated isolates have been rationally mutated using targeted molecular methods.
- Killed organisms. Probably the simplest sort of vaccine to produce. The organism is grown and then killed either chemically (e.g. with phenol or formaldehyde) or by heating.
- Component vaccines. The emergence of purified component vaccines has depended upon technological advances since the latter part of the 20th century. These have included advances in physical and chemical methods of separation as well as the development of recombinant DNA techniques, genome sequencing and bioinformatics (for the identification of prospective antigen genes in the genome).
- DNA vaccines. The antigen gene is cloned in a vector so that it is expressed from a promoter sequence that is functional in the host. Once the DNA is injected, the host expresses the desired antigen and then mounts an immune response.

Properties of a good vaccine:
- Stimulates an effective immune response
- It should elicit the correct balance of humoral and cell mediated responses
- The immune response should be directed to the relevant site within the host (e.g. the gut in the case of enteric infections)
- The immune response should be functional. For example, toxin neutralizing antibodies should be able to bind to toxin and neutralize its activity or if bacterial toxins are required antibodies should bind both the bacterium and complement.
- Is safe and does not cause adverse reactions
- Is expensive to manufacture and distribute
- Is stable
- Is easy to administer
- Should be simple for both manufacturer and regulatory authorities to control

Vaccines are made up of the antigens, adjuvant and excipients. Adjuvant is to enhance and modulate the immune response. Whereas exipients are buffers, salts, saccharides, proteins etc that maintain pH and osmolality of the vaccine.

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DNA vaccines. The antigen gene is cloned in a vector so that it is expressed from a promoter sequence that is functional in the host. Once the DNA is injected, the host expresses the desired antigen and then mounts an immune response.

Vaccines enhance adaptive immune responses to infection

Passive versus Active immunization

Current routine bacterial vaccination in the UK

<table>
<thead>
<tr>
<th>WHEN TO IMMUNISE</th>
<th>WHAT IS GIVEN</th>
<th>HOW IT IS GIVEN</th>
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<tbody>
<tr>
<td>2, 3 and 4 months old</td>
<td>Diphtheria, tetanus, pertussis (whooping cough), polio and Hib</td>
<td>One injection</td>
</tr>
<tr>
<td>Around 13 months old</td>
<td>Meningococcal (MenC)</td>
<td>One injection</td>
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<td>3 years and 4 months to 5 years old</td>
<td>Diphtheria, tetanus, pertussis (whooping cough) and polio</td>
<td>One injection</td>
</tr>
<tr>
<td>10 to 14 years old</td>
<td>BCG</td>
<td>Skin test, one injection, if needed</td>
</tr>
</tbody>
</table>

Quo: There is no vaccine against Serogroup B of N. meningitidis because

The bacterium is very aggressive
- Its antigens have not been isolated
- Its capsule is identical to a molecule present in normal humans
- Its genome is too large
- It has no capsule

Posted from: https://www.who.int/ncdc компаниелл/hiv1/hiv1/bgb/anltnsev/protection
<table>
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<tr>
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<td>5 years old</td>
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<td>BCG</td>
<td>Skin test, one injection, if needed</td>
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<tr>
<td>13 to 18 years old</td>
<td>Diphtheria, tetanus, polio</td>
<td>One injection</td>
</tr>
</tbody>
</table>

**DTP:** diphtheria, tetanus, pertussis (whooping cough)

- **Subunit vaccines:** antibodies neutralise toxins and block adhesion

**Conjugate vaccines** (carbohydrate)
- T cell recognition of protein carriers enhances B cell activation
- Promotes efficient antibody response to polysaccharide capsule

**Live attenuated vaccines**
- BCG gives some protection against tuberculosis in children but is ineffective against adult pulmonary disease
- New vaccine strategies based on genome information
- Very effective live attenuated vaccines are being developed for enteric pathogens (e.g. *Salmonella typhi*)

**Additional Information**

- Some bacteria produce polysaccharide capsules. These bacteria include organisms that colonise the nasopharynx, such as *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseria meningitidis*, which occasionally cause invasive infections like septicaemia and meningitis.