- Tuberculosis is caused by mycobacterium tuberculosis – an acid fast bacilli which contains a high concentration of lipids in the cell wall, the infection occurs after inhalation of an infected droplet. Tuberculosis predisposing factors = age malnutrition, alcoholism, immunodeficiency, and poor living conditions. Symptoms = chronic cough, fever, night sweats. Treatment – initial phase (2 months) – isoniazid, rifampicin, pyrazinamide and ethambutol. Continuation phase (4 months) – isoniazid, rifampicin. Monitoring = liver function test, renal function test and eye tests.
- Bronchitis – involves the production of mucopurulent sputum instead of usual mucoid and is caused by H.influenzae and S.pneumonia.
- Antimicrobial use: Advantages of combination therapy = reduction in the likelihood of drug resistance, provide synergy, improved survival, wide spectrum. Disadvantages = interaction, cost, side effects, and different pharmacokinetics.
- Viruses: are static structures so they have NO metabolic activity of their own and so they rely on the host biosynthetic machinery for protein synthesis. Viruses are not causes of spoilage. Viruses contain viral genome in a protein coat – either DNA or RNA. RNA viruses include HIV and influenza. DNA viruses include HepB and herpes simplex. Parasites include helminths and protozoa. Sulphonamides and trimethoprim inhibit folate synthesis and are inactive against anaerobes. Glycopeptides are good for gram positive. Rifampicin inhibits RNA synthesis and its used against gram positive organisms. Nitroimidazole inhibit DNA replication and are good for anaerobic organisms and protozoa. Linezolid – is an oxazolidinone antibacterial which is active against gram positive organisms and is used for MRSA and VRE.
- Tuberculosis is caused by mycobacterium tuberculosis (aerobic, acid fast due to waxy cell wall, bacillus). Risk factors of TB: extremes of age, HIV, cancer, diabetes, malnutrition, and end stage renal disease. Pulmonary TB cause haemoptysis – coughing up of blood. Treatment: sterilising antibiotics like rifampicin which kill actively growing and dormant bacteria, potentially bactericidal antibiotics like isoniazid and ethambutol which kill actively diving bacteria, and also bacteriostatic antibiotics. Rifampicin blocks RNA polymerase and induces CYP450. Steroids reduce fibrosis (scarring) during healing.
- C.difficile causes diarrhoea which can be treated using vancomycin. Diarrhoea can be caused by ciprofloxacin. Trimethoprim is used for UTI's. If a patient has shortness of breath – they may have pneumonia, but additional tests needed for diagnosis – chest x-ray, C-reactive protein, white blood count, urea and electrolytes, erythrocyte sedimentation rate. Other information needed = allergy status and details, if patient is pregnant, if the patient has had recent contact with animals, if the patient has travelled recently, if the cough is productive, antibiotics taken, full drug history and compliance to medicines.
- Key points patients need to understand about medication prior to discharge (counselling): emphasise the importance of completing the course of drugs, ensure the patient understands the dose and frequency to be taken, ensure the patient is compliant with their other medications, and explain the side effects.
- Contamination=transient presence of microbes. Colonisation = continuing presence of microbes. Infection = damage of tissues by microbes. Pathogenicity = ability of microbe to cause disease. Normal flora = microbes that co-exist with humans.
- Sepsis = uncontrolled systemic response to infection and can lead to organ dysfunction. Progress of clinical symptoms: SIRS (systemic inflammatory response system) to sepsis to severe sepsis to septic shock. Damage to epithelium due to bacterial infection causes loss of homeostasis and therefore can result in organ dysfunction. Stimulation of the host immune response by microbial toxins causes an inflammatory response that can lead to endothelial damage. Pro-inflammatory cytokines such as tumour necrosis factor (TNF), IL-1, and IL-6 are released in response to infection with the aim of destroying damaged tissue, and anti-inflammatory mediators like IL-10 and IL-13 are then released to restore homeostasis. In sepsis the imbalance between the pro-inflammatory cytokines and anti-inflammatory mediators result in: stimulated coagulation response, inhibited anticoagulant response, inhibited fibrinolytic response. These processes lead to endothelial damage and loss of equilibrium between coagulation and fibrinolysis which can lead to organ dysfunction. In sepsis the ability to down-regulate inflammatory response to infection is lost. Loss of immune regulation leads to SIRS and then CARS (excessive immunospression) through overexpression of anti-inflammatory mediators like IL-4 and IL-10. Role of endothelium = interaction with leukocytes, release of cytokines and inflammatory mediators, release of mediators of vasodilation and vasoconstriction and also clot formation. In sepsis: during the inflammatory response – endothelial cell activation causes vasodilation allowing leukocytes to access the infection site. Normally the endothelium down-regulates pro-inflammatory response by expressing anti-inflammatory and antibacterial mediators. In sepsis, failure of the endothelium regulatory function causes excessive vasodilation and inappropriate cytokine response. Initiation of coagulation occurs by the extrinsic and intrinsic pathway which leads to a common pathway. The extrinsic pathway is rapid and involves release of tissue factor into the plasma. The intrinsic pathway is slower and involves mediators that are already present in plasma. When the endothelial damage occurs the tissue factor from the extrinsic pathway activates coagulation factor 7, which then combines with and activates factor 10. Activated factor 10 combines with factor 5 in the presence of calcium ions to form prothrombinase. In the intrinsic pathway factor 12 is formed which activates factor 10 which leads to the formation of prothrombinase and calcium ions which catalyse the conversion of prothrombin to thrombin. Thrombin and calcium ions then catalyse the conversion of fibrinogen to fibrin – leading to clot formation. Homeostasis is the balance in physiological mechanisms like inflammation, coagulation and fibrinolysis. Severe sepsis is a procoagulant state associated with reduced fibrinolysis. Monitor the response to infection – fever, shock caused by hypotension and decreased renal perfusion), metabolic changes like increased carbohydrate metabolism and changes in cytokine production, and also malnutrition. Monitor white cell count, C reactive protein, erythrocyte sedimentation rate. Sepsis can cause meningitis, UTI's, cellulitis. Patients who develop sepsis are immunosuppressed. Signs of sepsis include high temperature, hypotension and tachycardia. Investigations for severe sepsis = FBC (RBC count, haemoglobin, haematocrit, WBC count, platelet count), clotting tests (pT, aPTT), arterial blood gas, serum electrolytes, glucose, LFT, urine analysis and ECG. Investigations for infection = skin, blood, urine, faeces, X-ray. Poor prognostic indicators: increased pT + aPTT + D-dimer and IL-6, low levels of antithrombin and patient may have thrombocytopenia.
- Resistance: resistance of a microorganism to clinically achievable concentration of a given antibiotic. There are 5 mechanisms of resistance: 1. Direct inactivation of antibiotic, 2. Modification of the cellular target, 3. Modification of the cellular target, 4. Modification of cell wall resulting in decreased antibiotic permeability, 4. Efflux of antibiotic from cell, 5. Changes in gene expression/metabolic bypass. These mechanisms may be innate/intrinsic to the cell or acquired. Innate or intrinsic resistance – is resistance which may be attributed to the normal structure or other properties of the cell, it includes factors that prevent uptake of the antibiotic such as impermeability of the cell AND also lack of cellular targets or altered target structure. Glycopeptides are large polar molecules that cannot pass through the outer membrane of gram negative organisms therefore gram negative bacteria are intrinsically resistant. Acquired resistance – is resistance in an organism that was previously susceptible to a particular antibiotic. It can arise through spontaneous mutation and horizontal gene transfer. Resistance to β-lactams may be acquired through: altered gene expression, alteration to target sites due to mutation of penicillin binding proteins, or changes in membrane permeability. Pseudomonas aeruginosa is resistant to β-lactams.
    1. Direct inactivation of antibiotics: AQUIRED RESISTANCE. Inactivation of β-lactam antibiotics like penicillins and cephalosporins by β-lactamase. B-lactamases cleave the β-lactam ring and inactivate the antibiotic. Most classes of β-lactamases use a serine ester hydrolase or a zinc ion to attack the ring. Chloramphenicol inhibits protein synthesis by preventing linkage of amino acids

5'-P groups enabling ligation (covalent linking of two ends of a DNA molecule using DNA ligase). Subsequent integration of HIV cDNA (complementary DNA) into the host involves viral integrase. Viral DNA is then spliced into the host genome. Integrase inhibitors – inhibit viral integrase and prevent DNA integration so the viral DNA will be circularised by host enzymes and degraded. Integrase inhibitor examples are Raltegravir (a 1-N –alkyl-5-hydroxy primidone) and is orally active and well tolerated. Insertion of viral DNA into the host genome is called strand transfer. Raltegravir inhibits strand transfer and is active against HIV-1 RNA and HIV-2 RNA. Raltegravir does NOT result in increased serum levels of total cholesterol, LDL cholesterol or triglycerides. Raltegravir side effects = nausea, vomiting and lipodystrophy. Raltegravir exhibits potent anti-retroviral activity when used with other HIV therapies.

- 4. Interference with protein modification by HIV protease inhibitors. Translation of viral RNA typically results in a long polyprotein precursor sequence that includes several essential proteins (1) like reverse transcriptase, integrase and protease (1). To become functional, the individual proteins MUST first be excised from the longer polypeptide chain (1). So cleavage of the polypeptide precursors into mature enzymes and structural proteins is mediated by the HIV protease and is therefore an essential step in the viral life cycle. Budded immature viral particles that contain catalytically inactive protease cannot undergo maturation to an infective form. HIV protease is a 99 amino acid aspartyl protease (1) which functions as a homodimer (2 identical substrates) (1) with only one active site (1). The two asparagine residues at position 25 in each subunit (1) are essential catalytic residues that activate a water molecule to hydrolytically cleave the polyprotein that binds between the two subunits (1). So asparagine is the active site of HIV-1 protease. Since the viral protease activity was shown to be essential to infection (1) there was an interest in protease as a potential target of new antivirals. The first protease inhibitor was introduced in 1996 (1). They function by binding in the tunnel/cleft between protease subunits (1) preventing correct interaction with substrates (1).
  Diagram of HIV-1 protease: (1)

Protease inhibitors do not inhibit host enzymes like trypsin or pepsin and therefore there is reduced toxicity (1). The function of protease inhibitors is to prevent post-translational modification of viral proteins leading to the production of non-infectious virus particles (1). When HIV-protease inhibitors are used in combination with other agents they bring about a dramatic clinical improvement and reduction in HIV related deaths. So they are very effective in combination with other drugs (1). Resistance occurs due to mutations around the active site (1) resulting in reduced affinity for inhibitors (1). Side effects = lipodystrophy (1) which is abnormal distribution of body fat (characterised by peripheral lipoatrophy, fat accumulation within the abdomen, hyperlipidaemia and insulin resistance (1)), high blood sugar and development of diabetes, liver toxicity, blood tests that monitor liver function as well as cholesterol and triglyceride levels are routine in patients on protease inhibitor therapy (1), high sugar/cholesterol is typical of protease inhibitors (1). Many protease inhibitors appear less active against HIV-2 protease (1) due to slightly different enzyme structures which causes reduced binding and therefore higher concentrations of protease inhibitors are needed. Some protease inhibitors can be used against hepatitis C (1). Protease inhibitors examples = Lopinavir, Amprenavir, Indinavir, Saquinavir.

- Interference with capsid maturation using maturation inhibitors. Prior to release from the host cell newly constructed virus particles undergo a maturation process – this is a key step in viral replication. Maturation of HIV virus particles, which occurs as they bud off from the infected cell, is triggered by the step-wise cleavage of the major viral structural polyprotein Pr55Gag to individual, mature Gag proteins which form the capsid. Maturation inhibitors cause the Gag protein and capsid to be defective and non-infectious. Current maturation inhibitors prevent the viral protease from processing Gag at one particular cleavage site. Maturation inhibitor examples = Bevirimat which disrupts capsid (Gag) protein processing and assembly specifically at CA-SP1 cleavage site which is between Gag coding for a 364 failures in clinical trials of Bevirimat occur due to polymorphisms in putative capsid protein cleavage sites preventing drug binding. Another example of HIV-1 maturation inhibitor = PF-46396.
- <u>Treatment of other viral infection considerations:</u> 1. therapeutic index which is concept that refers to the relationship between toxic and therapeutic dose. It determines how safe or toxic a drug is. 2. Severity of disease.
- <u>Herpes Virus:</u> is treated using nucleoside analogue-1 like Idoxuridine which was synthesised in 1959. It replaces thymidine in growing DNA chains, and can still form chains as it possesses –OH groups at 5 and 3 positions on sugar. Modification to organic base prevents binding to complementary base on DNA. It is too toxic to be used systemically, it cannot kill latent viruses and it only prevents replication. Nucleoside analogue-2 = acyclovir (acycloguanosine). Aciclovir (Zovirax) is a substituted guanine derivative. It lacks an –OH group at position 3 on the sugar so cannot accommodate chain elongation. Aciclovire is inactive and must be phosphorylated to form the nucleoside triphosphate in order to be used by DNA polymerase. Aciclovir is very selective low in cytotoxicity. Nucleoside isolated from a Caribbean sponge – cryptotethya crypta are the basis for synthesis of acyclovir. Aciclovir differs from previous nucleoside analogues in containing only a partial nucleoside structure – the sugar ring is replaced by an open chain structure. Activation of acyclovir: In the virus–infected cells aciclovir is phosphorylated by the viral enzyme thymidine kinase to form aciclovir monophosphate. Subsequent phosphorylation to di- and tri- phosphates is carried out by cellular kinases. In non-infected cells thymidine kinase does NOT exist and so aciclovir is not phosphorylated and remains inactive. Uses of aciclovir: used in treatment of herpes simplex infections of the skin and mucous membranes: HSV types 1 and 2, herpes simplex keratitis, Varicella Zoster (chicken pox and shingles). Aciclovir can be given orally, topically or IV infusion. Aciclovir is poorly water soluble and has poor bioavailability requiring IV administration to achieve high concentrations. pH of aciclovir solution is high (pH 11) and may lead to irritation at the site of infusion and because it is very insoluble absorption by mouth is poor and so high doses need to be administered. Valaciclovir is an esterified version of aciclovir and therefore has greater oral bioavailability. Famiclovir is also an oral prodrug which is converted by first pass metabolism to the antiviral drug penciclovir. Upon intracellular uptake, penciclovir is monophosphorylated by thymidine kinase and then converted to penciclovir triphosphate by cellular enzymes. Penciclovir triphosphate inhibits DNA polymerase of viruses but has no effect on cellular DNA polymerase. Penciclovir triphosphate has a lower affinity for viral DNA polymerase but a longer intracellular half life. Development of resistance: the virus may become thymidine kinase negative and so the drug will not become inactivated, the thymidine kinase may be altered in such a way that it does NOT activate aciclovir, the viral DNA polymerase may be altered so that it does not recognise the aciclovir triphosphate. Aciclovir is most effect against herpes simplex type 1. Nucleoside analogue-3: Ganciclovir is a nucleoside analogue of guanosine. Ganciclovir is phosphorylated to ganciclovir monophosphate by virus encoded thymidine kinase. The di- and tri- phosphates are produced by the actions of cellular enzymes. It is a more efficient substrate than aciclovir for HSV01 thymidine kinase so more ganciclovir triphosphate is produced compared to aciclovir triphosphate. Ganciclovir mode of action: ganciclovir triphosphate slows viral DNA replication however, short subgenomic