

BASIC MICROBIOLOGY

The Science of Micro-organisms

Single celled algae – bacteria – primitive fungi- viruses- protozoa

Antibiotics are effective against bacteria only

Some bacteria are **symbiotic** – benefits both the host and the organism eg E Coli produce Vitamin K

Some bacteria are **parasitic** – bacteria penetrate the protective barriers and cause harm

Some bacteria are **commensals** – live with the host and don't cause harm

Medical Microbiology deals with bacteria which are pathogenic Terminology

Genus – the first name of a bacteria – basic features including gram staining
Gram negative organisms – stain red

Gram positive organisms – stain blue violet

Species describe the characteristic of the bacteria – ie rod shaped, clusters, chains

Bacteria are Prokaryotes. Prokaryotes are cells with out a nuclear membrane. The DNA is free in the cytoplasm as a ring shaped double strand or chromosome. This chromosome contains all information necessary for bacterial life. Including storage of information, replication and control of metabolic processes

Bacterial cells also contain plasmids which are DNA particles which contain the memory for resistance

Mycoplasma and Chlamydia which are intracellular pathogens are also prokaryotes

Bacterial cell walls are thin, elastic permeable permitting exchange of nutrients and waste products as well as allowing entry of antibiotics
Some bacteria are surrounded by a bacteria capsule, which is made up of layers of polysaccharides and confers additional protection

Replication takes place when the DNA within the bacterial cell split by the gyrase enzyme. The daughter cell is produced and the original DNA strand is resealed. While the DNA strand is open, enzyme antibodies and genetic information which the cell needs is transferred via messenger RNA into the cytoplasm of the new cell

Cell growth takes place in 2 stages – Stage 1 the bacterium doubles its chromosome strand to guarantee transmission of genetic information to the daughter cell – Stage 2 a dividing wall develops in the bacterium dividing the cell into two. Bacteria can reproduce very quickly under the right conditions. Nutrients – Moisture- Oxygen-Temp 30-40 degrees C- pH value 6.8 -7.4 There are 4 distinct phases of growth. **Lag phase** cells increase in size but not in numbers. **Exponential phase** is high growth phase. **Stationary phase** nutrients depleted cell division slow- growth and death rate balanced. **Decline phase** cells die.

Aerobic bacteria only survive if oxygen is present

Anaerobic bacteria grow only in oxygen free conditions

Most of the medically important bacteria can survive in both conditions

Bacterial cultures are collected from blood, sputum, urine, and tissue and taken to the lab for culture at the same time trying to avoid contamination of the culture. Depending on the sample they will be analysed a number of ways The Micro will look at the size and shape and smell of the bacteria. The bacteria will then be grown on an agar /broth solution or some other medium in a Petrie dish- then gram staining may be done to distinguish between species. Gram positive organisms stain blue and Gram Negative Organisms stain red

Sometimes special cultures like blood agar will help identify the organism. E tests and oxoid discs are placed on the agar plate to determine which antibiotic will be effective against the organisms. The methods using disc diffusion are referred to as the **NCCLS , CDS and BSAC**. These tests are used to determine the MIC of an organism. Each disc has a different strength of drug in the disc and the concentrations are similar to those found in blood or urine. If bacteria continue to grow at the edge of the disc the organism is said to be resistant. If there is a clear zone around the disc this indicates the bacteria is sensitive to antibiotic The **MBC** (minimum bacterial concentration) is the minimum dilution of an antibiotic at which all bacteria are killed.

The **MPC** is the mutant prevention concentration. For this test large numbers of bacteria are placed on plates containing differing concentrations of antibiotic. By exposing the bug to sub therapeutic levels of antibiotic there is potential for bacterial to mutate. The concentration at which the antibiotic prevents mutant pathogens from occurring is called the MPC.

Sometimes **antibiotic assays** are performed. These are to determine the synergy between antibiotics, the level of drug needed to have a therapeutic effect and to make sure that drug levels will not be toxic

Immunologic testing may be done – this type of test detects the presence of bacterial antigens in CSF

Serologic Assays These are done to determine the presence of antibodies in the serum indicating whether or not disease has occurred.

Break Points This corresponds to the correlation between the achievable concentrations of an antibiotic in the blood and the MIC value or diameter of the zone of inhibition which has been determined

Eg If an antibiotic achieves 4 mg/l in the serum, then all pathogens with an MIC(**Minimum Inhibitory Concentration-90 - concentration at which 90% of all pathogens are killed**) below 4 mg/l can be regarded as being sensitive. But because most infection is found in the tissue not blood it is also useful to consider tissue penetration

Classification of Bacteria

3 Classes – Eubacterials - Actinomycetales – Spirochaetes (see pages for species

Which Bugs are where – Normal Flora

Micro organism	Skin	Mouth	Intestines	URT	Genital tract
Staphylococci	+++	+	+	++	++
Enterococci			++		+
Group a streptococcus	+	+++	+	+	+
Anaerobic cocci	+	+++	+++		+++
Pneumococci		+	+++	+	
Clostridia					
Enterobacteria	+	+	+++	+	+
Pseudomonas			+		
Haemophilus				+	+
Bacteroides		+++	+++	+++	+++
Mycoplasmas		++	+	+	++
Streptococci		++		+	

Pathogens – Disease causing organisms have a spectrum of infection.

Some infect multiple hosts some are very specific

A commensal bacterial becomes a pathogen when it penetrates an area where they are not normally located – this is termed an endogenous infection

An exogenous infection occurs when the bacterial pathogen does not come from the patient's own flora

Nosocomial Infections are those, which are caught while the patient is in hospital. These are often resistant to antibiotics as they are constantly exposed to them.

Which Bugs cause what infection?

Micro organism	Skin Bone	Sepsis	Intestinal	RTI Ear	UTI	Genital tract	Noso comial
E.coli			+		+		
Enterobacter cloacae	+	+		+	+		+
Serratia marascens	+	+		+	+		+
Salmonella typhi			+				
Haemophilus influenzae				+			
Campylobacter pylori			+				
Chlamydia trachomatis					+		
Nesseria gonorrhoeae						+	
Moraxella catarrhalis				+			
Klebsiella pneumoniae				+			
Pseudomonas aeruginosa	+				+		+
Streptococcus pneumoniae				+			
Streptococcus pyogenes	+			+			
Stapylococcus aureus	+			+			+
Staphylococcus epidermidis	+						

Infection and Infectious Diseases

Infection an attack by pathogens, adhesion to tissue and reproduction of the pathogens

Inflammation defence reaction of the body and not always to an attack by pathogens

Preconditions for Infection – Exposure –susceptibility of the host and penetrability of the pathogen

Infectious Disease occurs if a natural barrier such as skin, or mucous membrane is penetrated

If the strain of pathogen is very virulent

If a large number of pathogens penetrate barriers

The hosts natural defence system is strong

Inflammatory Diseases are often caused by an infection

Can be Acute- Chronic or Purulent . Purulent is when white blood cells migrate from the blood vessels into the tissue. They destroy the bacteria by absorbing them into the cell . This is called phagocytosis. When the phagocytes die, pus is formed. At this point extra fluid may leak out of blood vessels into the tissues causing swelling and sensitisation of nerve endings

Bacteria sometimes cause an infection by excreting toxins eg
Corynebacterium diphtheriae

Clinical Signs of Infectious Disease –raised temperature – increased ESR, increase in number of white cells- swelling of lymph nodes- redness of skin or mucous membranes – general fatigue and pain

Routes of Transmission of Infection

Droplets (human contact)

Insect Bites – Mosquito -

Consumption of contaminated water or food eg Salmonella typhi, E.coli

Animal contact - eg Pasteurella Multicida

Bacteraemia – temporary presence of bacteria in the blood which does not at first result in disease

Septicaemia – pathogens continuously or intermittently enter the blood stream from a focus of infection ie urine 50% or pus in lungs – very serious infection – Can lead to septic shock where the pathogens release endotoxins which cause an exaggerated immune response in the patient and gradually organs shut down. Gram negative organisms lead more quickly to septic shock

Infectious Diseases treated by Ciproxin and Avelox include the following:

Avelox – Mixed Gram Positive, Gram Negative and Atypical pathogens	Ciproxin Gram Negatives
Sinusitis	Ear Infections - Pinna of Ear
Acute exacerbations of Chronic Bronchitis	AECB/Pneumonia if Pseudomonas present
Pneumonia – Lobar, Broncho and Atypical	UTI / Pyelonephritis
Skin but not indicated yet	Prostatitis
Gynaecological infections (future)	Gastro intestinal infections- Salmonella
	Bacteraemia and Septicaemia
	Skin (indicated but Avelox better)
	Bone and Joint

Defence Mechanisms of the Human Body.

- Skin
- Mucous Membranes – action of cilia
- Changes in pH – acid mediums
- Commensal organisms
- Irrigation by secretions
- White blood cells – macrophages carrying out phagocytosis (Some bugs like Legionella can survive in a macrophage which is why they are so hard to treat and can cause fatal infections
- The Complement system – Proteins which damage bacterial cell membranes – cytolysis or lysozymes a protein which is released when a phagocyte decomposes, acting on the wall of the bacteria inside the phagocyte – present in bronchial mucous and tear fluid
- Antigens and antibodies – Antibodies bind the antigens that have penetrated the bacteria making them ineffective
- B lymphocytes which are produced in the bone marrow stem cells produce the antibodies approximately 2 – 3 weeks after being in contact with an antigen
- Antibodies are immunoglobulins
- Some lymphocytes with this memory for and antigen are produced in the Thymus. The are called T lymphocytes
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- Immunodeficiencies and Weaknesses
- Reduction or loss of function of the T cells or B cells
- Disturbances in the activity of the macrophages

Antibiotics

Anti bacterial substances that interfere with the life and reproductive cycles of the micro organism

Antibiotics are bacteriostatic (Arrest of growth)or bactericidal (Destruction of cell)

Effective bactericidal action is defined as killing 99.0% of organisms within the first few hours after administration

Quinolones – Cephalosporins – Penicillins are Bactericidal

The combined use of a bacteriostatic and bactericidal antibiotic can lead to a loss of effect if the bacteriostatic antibiotic inhibits the reproduction of bacterial which is necessary for the effect of the bactericidal antibiotic

Antibiotic Mode of Action

Antibiotics work either on the bacterial cell wall or interfere with metabolic processes

Macrolides, Chloramphenicol, Aminoglycosides and Tetracyclines inhibit protein synthesis

Quinolones, Rifampicin and Metronidazole are nucleic acid synthesis inhibitors

Trimethoprim and Sulfonamides inhibits folic acid metabolism

Penicillins Vancomycin and Cephalosporins inhibit the development of a strong framework in the cell wall (cell wall synthesis) and pressure inside the cell causes the bacterium to burst

Cytoplasmic Membrane – Beta Lactams and Imidazoles

Choosing an Antibiotic

Depends on:

Pathogens isolated on culture or suspected – these have been determined by clinical trials in the past

Resistance patterns in community or hospital

Primary Illness

Cost

Spectrum of Activity

Route of Administration

Metabolism

Side Effects

Risk factors the patient may have diminished renal function, diabetes mellitus, old age, exposure to certain environmental factors, be immune-compromised, taking drugs which affecting absorption of antibiotic, previous anti-microbial therapy, recent hospitalisation and exposure to resistant pathogens, overweight.

Pharmacokinetics and Pharmacodynamics of the antibiotic – eg drug interactions, drug toxicity, form of administration, dose, absorption, volume of distribution, tissue penetration, side effects, metabolism, spectrum of activity
Pharmacodynamics – the study of the relationship between the concentration of the drug in the serum or at the site of infection and the biological effects of the drug (bacterial killing , growth inhibiting or affecting virulence characteristics.)

Pharmacokinetics - the relationship between the dose of the drug and the concentration of the drug in serum and at the site of infection over time

The parameter that best equates with bacteriological eradication is time above MIC

Anti microbials with concentration dependent bacterial killing patterns and prolonged effects against pathogens show in in vitro studies the best correlation with bacteriological eradication and the prevention of the development of resistance

Quinolones have concentration dependent killing patterns

**Antibiotics Commonly used in Avelox and Ciproxin Targeted Infections
According to Australian Therapeutic Guidelines**

Acute Bacterial Sinusitis	Amoxycycline, Augmentin, Cefuroxime, Cefaclor, Doxycycline
Acute Exacerbations of Chronic Bronchitis	Amoxicillin, Doxycycline
Community Acquired Pneumonia	Amoxicillin, Roxithromycin, Moxifloxacin Benzyl Penicillin, Cefuroxime, Ceftriaxone, Doxycycline, Gentamicin Erythromycin, Timentin, Piperacillin, Imipenem or Meropenem
Nosocomial Pneumonia	Depends where the infection originates. Mostly from UTI so would use drugs indicated there But add Ciproxin and Imipenem
Ventilator Associated Pneumonia	Same as for Nosocomial Pneumonia but likely to use Ciproxin or Imipenem or Meropenem earlier
Sepsis	Di/Flucloxacillin, Amoxicillin, Ampicillin, Gentamicin, Piperacillin, Timentin, Ceftriaxone, Metranidazole, Vancomycin, Ciproxin (Pseudomonas) Benzyl penicillin, Clindamycin
Skin Infections	Benzyl Penicillin, Metronidazole, Timentin, Ciproxin, Doxycycline, Piperacillin, Di/Flucloxacillin, Clindamycin, Cephalexin, Vancomycin
Bone and Joint Infections	Di/flucloxacillin – empiric for children cefotaxime or ceftriaxone, cephalothin, cephalozin, clindamycin
Gastrointestinal Infections	Erythromycin, Norfloxacin, Ciprofloxacin, Doxycycline, Amoxicillin, Trimethoprim, Ampicillin, Metronidazole
Urinary Tract Infections	Trimethoprim, Cephalexin, Augmentin, Nitrofurantoin, Gentamicin, Ceftriaxone
Prostatitis	Ampicillin, Genatmicin, Norfloxacin, Ciprofloxacin, Doxycycline, Trimethoprim

Which Drugs Treat Which Bugs

Pathogen	Drugs
E.coli	Bactrim, Augmentin, Cefuroxime Ciproxin, Avelox
Klebsiella pneumoniae	As above
Proteus	As above
Enterobacter	As above
Salmonella	Ciproxin, Chloramphenicol and Bactrim
Pseudomonas	3rd gen cephalosporins(ceftriaxone/ceftazidime) Imipenem and Meropenem, Aminoglycosides and Ciproxin
Haemophilus	Bactrim, Tetracyclines. Azithromycin, Augmentin, Cefuroxime, Ciproxin and Avelox
Neisseria gonorrhoeae	Ceftriaxone or Ciproxin
Neisseria meningitidis	IV penicillin, ampicillin, rifampicin, Ciproxin
Bacteroides	Flagyll, Avelox
Mycobacteria	Flagyll, Bactrim, Augmentin, Clindamycin, Avelox add Rifampicin and or Isoniazid
Chlamydia	Erythromycin, Doxycycline, Avelox Rulide, Azithromycin or Clarithromycin
Mycoplasma	As above
Legionella	As above but add Rifampicin

Resistance

Many bacterial develop mechanisms by which they can survive in the presence of antibiotics- they become resistant

Forms of resistance –

- Natural – genetically determined to be insensitive to an antibiotic
- Mutational accidental or experimental changes in bacterial DNA
- Multi drug resistant- bacteria resistant to several classes of drug
- Cross Resistance – bacteria resistant to drugs in one class or group
- Transferable – plasmid mediated
- Primary resistance – the bug is resistant to the drug before even starting therapy
- Secondary resistance – arises during therapy where a previously susceptible pathogen become resistance

Mechanisms of Resistance

Penetration Barriers – Membrane structures of the bacteria changed so that the antibiotic can no longer penetrate

Active Efflux – The drug enters the cell but is pumped out again

Changes in Target Structure- the structure the antibiotic targets eg an enzyme is changed and the antibiotic no longer recognises the structure

Destruction of the Antibiotic by enzymes (Beta lactamases) MOST WIDELY SPREAD AND MOST IMPORTANT MECHANISM

Types of Resistance Development

One step – One contact with the pathogen, a single mutation occurs and the antibiotic no longer works

Multiple step –repeated contact with the bacterium, needing several mutations.

ESCAPM Pathogens

Enterobacter, Serratia, Citrobacter, Acinetobacter, Proteus, Morganella Pathogens that produce a beta lactamase to inactivate the antibiotic – Ampicillin – Cefoxitin and Imipenem strongly induce the production of this enzyme

ESBL'S

E coli, Klebsiella, and Proteus species which produce enzymes capable of destroying the efficacy of extended spectrum cephalosporins

VRE Vancomycin resistant Enterococci - Plasmid mediated resistance

Mechanisms of Bacterial Resistance by Class of Antibiotic

CELL WALL SYNTHESIS INHIBITOR	
Beta Lactams – Penicillins, cephalosporins carbapenems eg Amoxil. Augmentin Cefotaxime, Imipenem, Meropenem	In activation by beta lactamase enzymes which hydrolyse the beta lactam ring in the structure of the antibiotic Outer membrane of some gram negative bacteria are intrinsically resistant
Glycopeptides Eg Vancomycin and Teicoplanin	Alteration in the target site of the antibiotic so that it cannot bind and exert activity
NUCLEIC ACID SYNTHESIS INHIBITOR	
Quinolones eg Ciproxin and Moxifloxacin	Mutation of chromosomal genes parC and par E for topoisomerase 4 and gyrA and gyr B for DNA gyrase Efflux pump mechanisms for Ciproxin Chromosomal mutations that result in fewer porins in the bacterial cell membrane leading to reduced entry of the antibiotic
Mupirocin	Not known
Lincosasmides eg Clindamycin	Not known
PROTEIN SYNTHESIS INHIBITOR	
Aminoglycosides eg gentamicin, amikacin, tobramycin, streptomycin	Drug Inactivation, modification of aminoglycoside by acetylation preventing anti microbial activity
Macrolides eg Erythromycin, Clarithromycin, Azithromycin	Alteration of ribosomes preventing the macrolide from binding
Tetracyclines eg tetracycline Doxycycline	Efflux pump mechanisms
Chloramphenicol	Drug inactivation by acetylation
Ketolides eg Telithromycin (not registered yet)	Mutations and Modifications of RNA preventing drug from binding
Oxazolidinones eg Linezolid	Single nucleotide change in gene encoding RNA
TETRAHYDROFOLIC ACID SYNTHESIS INHIBITORS	
Trimethoprim and Sulphonamides	Bacteria deliberately overproduce and enzyme which saturates the antibiotic but still allows folic acid to be catalysed

QUINOLONE RESISTANCE

Quinolone mode of action is different and with the latest generation of quinolones resistance will be much slower to develop

Resistance develops through chromosomal mutation mutations in DNA gyrase A and B and in Topoisomerase 4 and is not acquired through plasmids

There is also evidence that the earlier quinolones are affected by efflux pump mechanisms and decreased permeability in the cell wall
Later quinolones do not seem to be affected by efflux pump mechanisms

Quinolones are synthetic agents so there is no natural resistance