

# Drug Resistance

## Lecture 5

# Resistance

There are two basic types of antimicrobial resistance.

**Intrinsic resistance** – i.e. inherent or natural resistance

(e.g. *Chlamydia* do not have peptidoglycan and so are not susceptible to  $\beta$ -lactams)

**Acquired resistance** - resulting from alteration of the bacterial genome.

Alteration of the bacterial genome can occur through “**vertical evolution**” (Darwinian-type) and “**horizontal evolution**” (transfer of genetic elements between microbes through various mechanisms)

- **Transposons** (small mobile ‘bits’ of DNA that can move around the gene or to other genes within the same cell)
- **Plasmids** (circular, double-stranded units of DNA that replicate independently of the chromosomal DNA)

# Resistance mechanisms

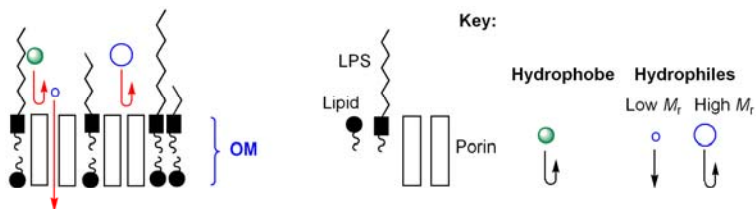
Bacteria have various **mechanisms** by which they can **exhibit (show), or develop, resistance to antibiotics**, for example:

- **Exclusion of the drug**
  - The outer membrane in Gram-negative bacteria is an extra barrier
- **Enzymatic degradation of the drug**
  - Conversion of the drug to an inactive species
- **Modification of the drug target**
  - The bacteria changes the structure of drug targets so they are insensitive to the drug
- **Efflux mechanisms**
  - The bacteria can 'pump out' the drug as soon as it enters the cell
- **Enhanced production of the target to compensate**
  - The bacteria can up-regulate the production of key molecules
- **By-pass of the target**
  - The bacteria can use an 'alternative route' to make key molecules

2

## The outer membrane of GNB

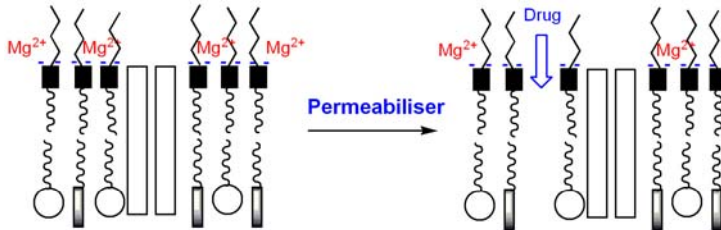
**Exclusion of the antibiotic:** The outer membrane (OM) is an **additional barrier** to extracellular solutes, including antibacterial agents. This is general feature of intrinsic (natural) resistance of GNB, and of mycobacteria, to many antibiotics.



The outer membrane (OM) in GNB presents a barrier which prevents passage of large or hydrophobic agents into the bacterial cell. In many GNB, phospholipids are absent from the OM; in consequence, **large (e.g. glycopeptides) and hydrophobic agents (e.g. macrolides, rifamycins) cannot readily penetrate and diffuse across the OM.** **Small, hydrophilic solutes can pass through the OM** through aqueous channels/pores formed by transmembrane proteins (*porins*). <sup>3</sup>

# Disruption of the OM in GNB

The surface of the OM is **anionic** overall due to an excess of negatively-charged head-groups in LPS over positively charged groups. The anionic sites are neutralised by counter-cations, e.g.  $Mg^{2+}$ . The **cations stabilise the OM by chelation** of LPS-LPS and LPS-protein molecules.

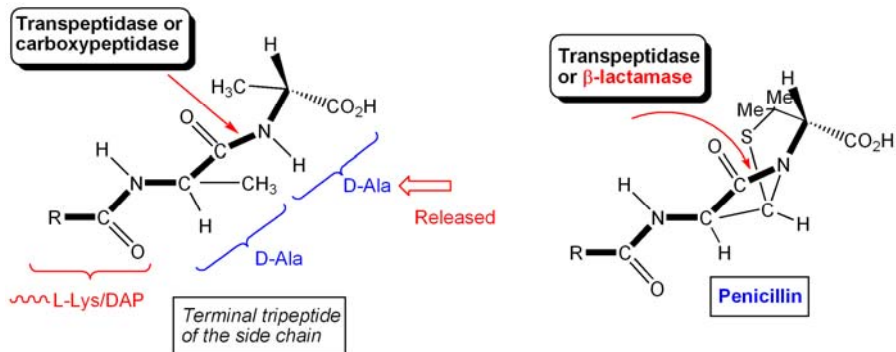


Removal or displacement of the cations destabilises, disorganises, permeabilises and **sensitises the OM** through coulombic repulsion and altered packing of the membrane components. As a consequence, large and hydrophobic molecules (e.g. lysozyme, antiseptics, antibiotics) can penetrate the OM and gain access to their targets.

4

# Penicillins: mode of action

Penicillins irreversibly bind to (deactivate) the transpeptidase enzymes so stopping the process of peptidoglycan cross linking.



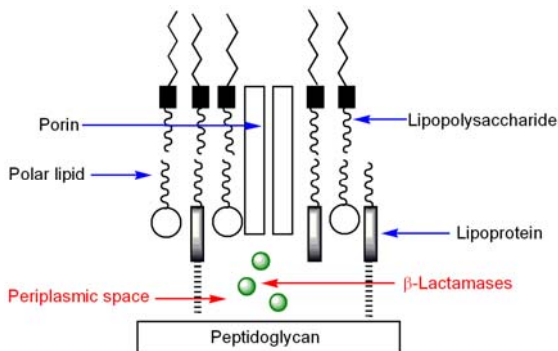
5

# Degradation of penicillins

**$\beta$ -Lactamases** (penicillinases) are enzymes that hydrolyse the cyclic amide bond of  $\beta$ -lactams and prevent them binding to penicillin binding proteins, e.g. transpeptidases. There are different sorts of  $\beta$ -lactamases; GPB produce basically one  $\beta$ -lactamase, but GNB produce wide range of variants:

**for GNB:** (see figure)  
**cell-bound:** found in the periplasmic space, i.e. a strategic location.

**for GPB:**  
**inducible:** produced in response to a threat (economical for bacteria)  
**extracellular:** a long-range action, but wastage by dilution.



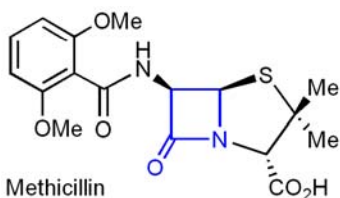
6

# Overcoming $\beta$ -lactamases

**What solutions are there to the  $\beta$ -lactamase problem?**

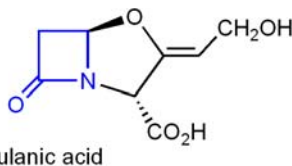
**Develop resistant  $\beta$ -lactams**

e.g. **methicillin**:  $\beta$ -Lactamase resistance is associated with increased steric hindrance about the  $\alpha$ -C to the amide link.



**Inhibit the  $\beta$ -lactamases with another drug**

e.g. by **clavulanic acid** (isolated from *Streptomyces spp.*). This has little antibacterial activity (by binding to transpeptidases) but causes irreversible acylation of the  $\beta$ -lactamase enzymes.

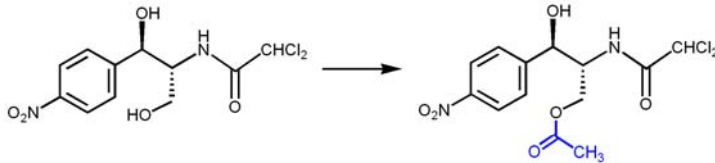


7

# Enzyme inactivation of the drug

Other examples of antimicrobial agents which are enzymically modified (inactivated) by their target bacteria are:

**Chloramphenicol** : resistance is most often due to enzymic acetylation of the drug by chloramphenicol acetyl transferase (CAT).

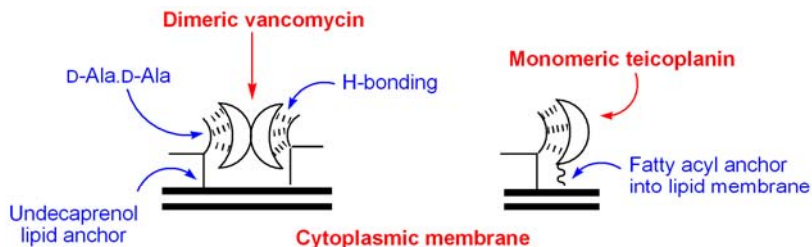


**Aminoglycosides** : these may be modified to render the antibiotic inactive, e.g. acetylated by an *acetyltransferase*, phosphorylated by a *phosphotransferase* or by conjugation with a nucleotide.

8

# Glycopeptides: mode of action

The glycopeptide antibiotics inhibit late stages of peptidoglycan synthesis involving transfer of completed, lipid-bound precursor units from the cytoplasmic membrane to the growing cell wall



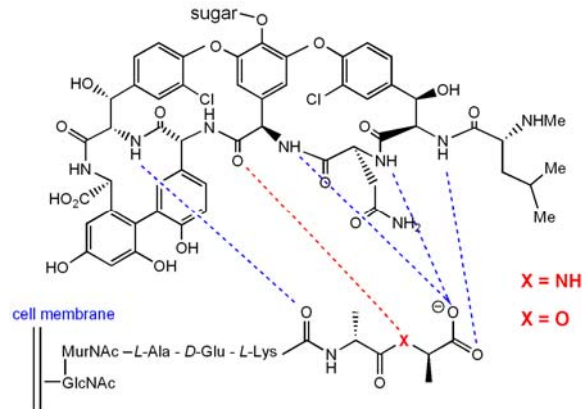
High-affinity binding of glycopeptides via the "chelate effect"

9

# Glycopeptides: modification of the target

There is **intrinsic resistance** in GNB (due to OM) and *some* GPB, but also **acquired resistance** in *Enterococcus faecium*.

Resistance to vancomycin and teicoplanin is acquired from a plasmid, resulting in the production of 7 new polypeptides, 3 of which confer resistance through the formation of a **modified peptidoglycan precursor**.



10

## Resistance to glycopeptides

The replacement of **D-Ala.D-Ala** by **D-Ala.D-Lac** (Lac = lactic acid where the NH<sub>2</sub> is replaced by an OH) results in **loss of one of the H-bonds** critical for binding of vancomycin or teicoplanin. Affinity for the antibiotic decreases ~100x, and transglycosylation or transpeptidation are no longer inhibited.

The **Lac** residue is lost (*as is the terminal D-Ala of the normal precursor*) during cross-linking or through the action of a carboxypeptidase, and therefore **Lac** is *not* found in the mature peptidoglycan.

Organisms with **intrinsic** resistance to glycopeptides have precursors similar or identical to **X**, explaining their resistance. The prevalence of *E. faecium* and of VRE (Vancomycin-Resistant Enterococcus) and multidrug resistant strains is rising.

11

# Modification of the drug target

Other examples where the **antibiotic target is modified** to prevent the high affinity binding of a drug are given below:

Resistance to **trimethoprim**, an antifolate antibacterial, is displayed by alterations in dihydrofolate reductase (DHFR) - the target enzyme.

**Quinolone resistance** is effected by mutations in topoisomerase IV (DNA gyrase), which reduces the binding affinity of the drug to its target. Resistance in GNB has also been ascribed to resistance genes (from plasmids) which produce proteins that bind to the topoisomerase and so “protect it” from the action of the drug.

**Aminoglycoside resistance** can result from modifications of the structure of the bacterial ribosome.

12

# Bypass of drug target

## **Penicillins:**

**Methicillin** resistance, in MRSA, results from the production of an additional transpeptidase (PBP2', or PBP2a) which is not susceptible to inhibition by penicillins.

## **Antifolates:**

**Sulfonamide antibacterials** are rendered ineffective in many cases as the bacteria have evolved an alternative route to folic acid which does not need the dihydropteroate synthase enzyme (DHPS).

Resistance to **Trimethoprim** also results from an overproduction of the target enzyme dihydrofolate reductase (DHFR).

dihydropteridine diphosphate +  
*p*-aminobenzoate

DHPS

dihydropteroic acid

dihydrofolic acid

DHFR

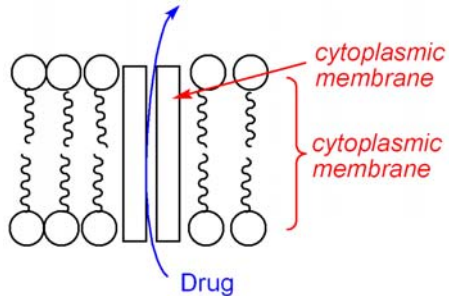
tetrahydrofolic acid

13

# Efflux mechanisms

**Efflux pumps**, or transporters, are proteins localized in the cytoplasmic membrane. They can remove a drug from a bacterial cell, so **lowering the intracellular concentration** of a drug.

Efflux pumps are “**active transporters**” as they require a source of energy to perform their function (e.g. ATP, or a transport which is coupled to a potential difference across the membrane). There are many sorts of efflux pumps, but which fall into one of five groups based on the protein sequence and energy source used.

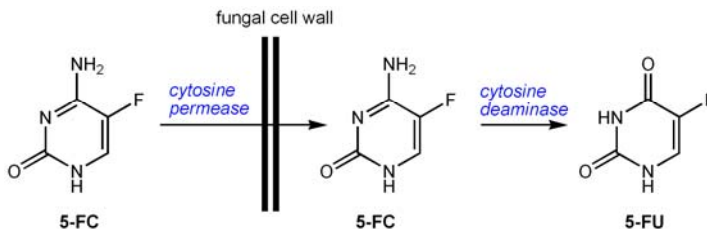


Bacterial resistance to drugs **by this mechanism** has arisen in the case of tetracyclines, macrolides, quinolones and chloramphenicol.

14

# Resistance 5-fluorocytosine

The activity of the **permease** and **deaminase** enzyme are believed to be an important factor in determining **the spectrum of activity** of 5-FC as well as **incidences of secondary resistance**. (remember 5-FU cannot be used as the drug as permease activity for uracil is low.



The low activity of permease enzymes in humans has been attributed to the relatively low toxic side effects of 5-FC. The intestinal disturbance noticed by some patients is believed to be due to conversion of 5-FC to 5-FU by a deaminase enzyme in gut bacteria. Combination therapy can be used to help the problem of the development of resistant strains.

15

# Resistance to fluconazole

Resistance of the yeast *Candida albicans* to fluconazole has manifested itself in a wide variety of ways:

***Point mutations in the ERG11 gene (encoding lanosterol 14 $\alpha$ -demethylase)***

Reduced drug affinity for the target enzyme

Over-expression of enzyme, as revealed in increased ergosterol synthesis

***Alterations in other enzymes of the ergosterol biosynthetic pathway***

Production of various sterols supporting growth;

Cross-resistance to other azoles and amphotericin B (AmB)

***Overexpression of CDR and MDR genes encoding efflux pumps***

Reduced drug accumulation in the cell

***Mechanisms to be defined***

Variations in plasma membrane components and altered cell wall proteins