Bacterial Resistance of Antibiotics - II

Inhibitors of peptidoglycan synthesis

β-Lactams

Acquired resistance to β-Lactam antibiotics can occur by three different mechanisms: **inactivation of the antibiotic, alteration of the target site and reduced permeability**.

A first mechanism: β-Lactams are inactivated by enzymes called β-lactamases which hydrolyze the cyclic amide bond in the antibiotic molecule. **Penicillins are converted to penicilloic acid which is unable to bind to penicillin-binding proteins (PBPs).** A similar reaction occurs with **cephalosporins**, *except that the cephalosporoic acid derivative is unstable and tends to break up*.

It is worth noting that in **Gram-negative** organisms, **β-lactamases are found in the periplasmic space where they inactivate β-lactams before the antibiotics can bind to their PBP targets on the cytoplasmic membrane**. In **Gram-positive** organisms, however, **β-lactamases are excreted extracellularly and therefore resistance is very much a characteristic of the population rather than individual β-lactamase-producing cells**. *If enough enzyme is synthesized, levels of β-lactam may be reduced sufficiently to permit growth of non-β-lactamase-producing strains.*

A second mechanism of resistance involves **alterations in PBPs which affect binding of β-lactams.** These changes have been found to occur by multiple substitutions through recombination rather than point mutations. Clinically, one of the most important examples of β-lactam resistance is that found in **methicillin-resistant *Staph. aureus* (MRSA)** strains. These are causing increasing concern in hospitals, especially because methicillin resistance is often accompanied by multiple resistance to unrelated antibiotics. Methicillin is resistant to β-lactamases and is a mainstay in the

treatment of *Staph. aureus* since over 90% of hospital strains produce β-lactamase. Methicillin resistance is due to a novel PBP with low affinity for β-lactams. It is capable of functioning when all other PBPs have been inhibited and is sufficient to catalyze all the reactions necessary for cell growth.

A third resistance mechanism is akin to that described for the AGAC antibiotics and chloramphenicol, **whereby changes in the outer membrane porins of Gram-negative bacteria reduce the penetration of β-lactams resulting in low levels of resistance.**

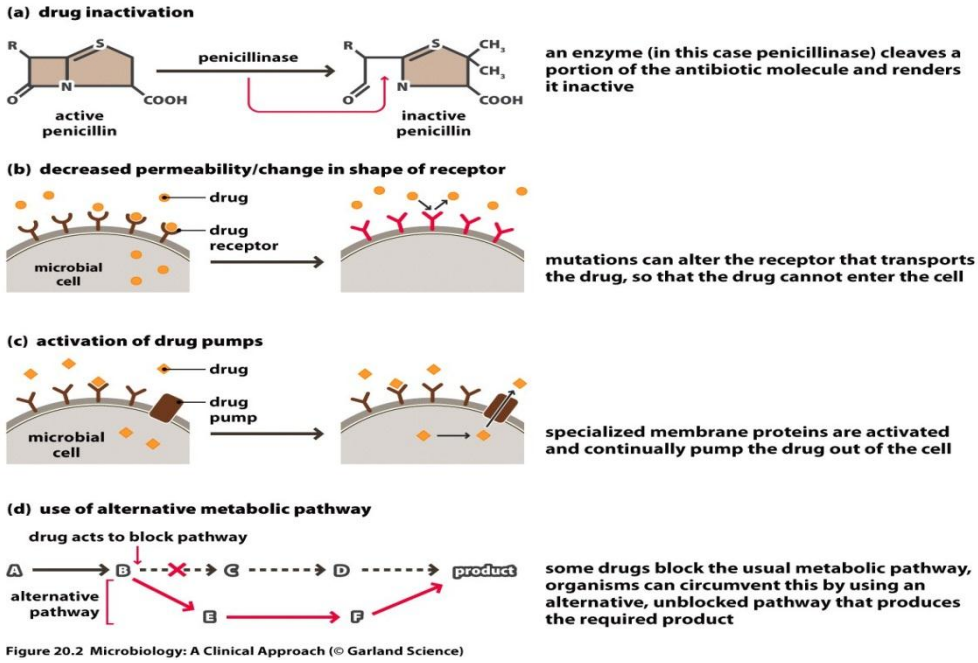
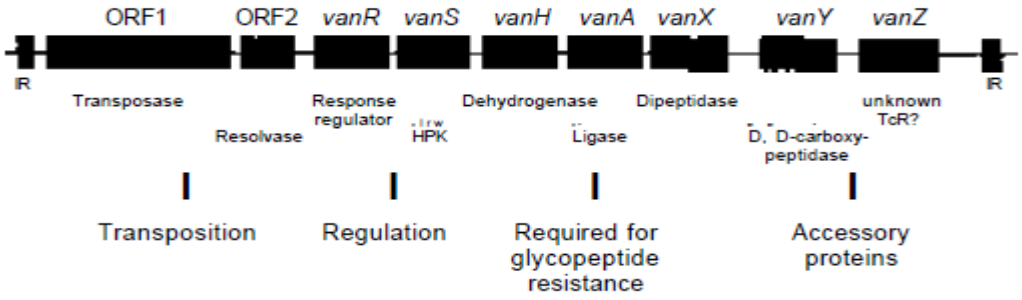


Figure 20.2 Microbiology: A Clinical Approach (© Garland Science)

Glycopeptides

Glycopeptide antibiotics interfere with **peptidoglycan synthesis** by binding to the D-alanyl - D-alanine terminus of peptidoglycan precursors. **Resistance to glycopeptides was thought unlikely** *because the changes in integral structures and functions of the cell wall and the enzymes involved in its synthesis would render bacteria non-viable*.



Organization of glycopeptide-resistance genes in transposon Tn1546. IR, invested repeats; HPK, histidine protein kinase; TcR, low level teicoplanin resistance.

Acquired resistance to the glycopeptides is transposon-mediated and has so far been largely confined to the enterococci. Two types of acquired glycopeptide resistance have been described: The **Van A phenotype** is resistant to vancomycin and teicoplanin, whereas **Van B** is resistant to vancomycin only. Van A and Van H are essential for the expression or resistance, which is due to a modification of the peptidoglycan pathway to produce precursors with reduced affinity for glycopeptides. Van A is a ligase which catalyzes the synthesis of D-alanyl-D-lactate depsipeptide instead of D-alanyl-D-alanine. Van H is a dehydrogenase which catalyzes the synthesis of D-lactate as the substrate for Van A.

Fosfomycin

Fosfomycin inhibits **pyruvil transferase**, which is an enzyme involved in peptidoglycan synthesis. Two mechanisms of acquired resistance have been described for fosfomycin .

Plasmid- or transposon-mediated resistance occurs by inactivation of the antibiotic. Fosfomycin is combined with glutathione intracellularly to produce a compound lacking in antibacterial activity.

A second mechanism of acquired resistance to fosfomycin involves chromosomal mutations in sugar phosphate uptake pathways which are responsible for transporting fosfomycin into the cell. The alterations decrease accumulation of the antibiotic to levels below those required for inhibition.

Membrane-active antibiotics

Polymyxins

Polymyxins are a group of antibiotics which disrupt bacterial cell membranes. Two mechanisms of acquired resistance to the polymyxins have been identified.

Acquired resistance to polymyxins in *E. coli* occurs because of **chromosomal mutations which cause incorporation of aminoethanol and aminocarabinose in lipopolysaccharide (LPS) in place of phosphate groups.** The altered LPS has a decreased ionic charge which results in lowered binding of polymyxin and thus an increase in resistance to this group of antibiotics.

The mechanism of acquired resistance in *Pseudomonas aeruginosa* is different. **Chromosomal mutations result in the increase of a specific outer membrane protein with a concomitant reduction in divalent cations**. Polymyxins bind to the outer membrane at sites normally occupied by divalent cations, and therefore it is thought that a reduction in these sites will lead to decreased binding of the antibiotic with a consequent decreased susceptibility of the cell.

Multidrug resistance pumps

Acquired low-level resistance to many unrelated antibiotics by efflux has also increased in prominence in recent years. For example, **MAR** (multiple antibiotic resistance) mutants were first described in the early 1990s in *E. coli* and were resistant to low levels of chloramphenicol, tetracyclines, rifampicin, penicillins and quinolones, due to impaired uptake of the antibiotics. **Increased active efflux** of the drugs has been shown to be important in this type of resistance. A number of multidrug resistance pumps (**MDRs**) have been identified and are widespread among bacteria. For example, seven distinct MDRs have been described in *E. coli* alone.

The most common type belongs to a group of proteins involved in membrane translocation. This type of MDR is closely related to **specific efflux proteins** such as that responsible for tetracycline resistance. The origins of MDRs are unknown but a number of factors suggest that they may have arisen by mutations in specific drug efflux pumps causing a loss of specificity. These factors include **the similarity of some MDRs to specific drug efflux pumps such as tetracycline**, and **the high incidence of apparently independent evolution of MDRs.**

Antibiotics with other resistance mechanisms

Bacitracin

Acquired resistance to bacitracin has been observed in laboratory strains of *Staph. aureus,* but resistance has been unstable and no resistant mutants have yet been isolated *in vivo.* Gram-negative bacteria are **intrinsically resistant** to bacitracin, which **inhibits the transfer of pentapeptide units to peptidoglycan.**

Antimycobacterial drugs

The advent of multidrug resistant strains of *Mycobacterium tuberculosis* (**MDR-TB)** has led to increased fears of untreatable infections by serious pathogens. **Rifampicin**, **streptomycin** and, occasionally, the **quinolones** are drugs used in the treatment of mycobacterial. There are some drugs, important in the antimicrobial treatment of tuberculosis, where resistance has arisen in MDR-TB strains, but where the mechanisms are unclear. These drugs include **isoniazid**, **pyrazinamide** and **ethambutol**.

There are two mechanisms of acquired resistance to isoniazid which have been proposed. **The first suggests that mutations in the *katG* gene inhibit the metabolism of isoniazid into an active form which inhibits an essential protein, InhA, in mycobacteria.**

Pyrazinamide is a structural analogue of isoniazid and is **converted to the active acid derivative intracellularly by a nicotinamidase.** Pyrazinamide resistance has been linked to **reduced levels of nicotinamidase.**

Mutations resulting in **ethambutol** resistance can arise spontaneously. The exact changes are unknown but may **involve enzymes in carbohydrate synthesis pathways.**