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Antimicrobial resistance mechanisms fall into four main categories: (1) limiting uptake of a drug; (2) modifying a drug target; (3) inactivating a drug; (4) active drug efflux. Intrinsic resistance may make use of limiting uptake, drug inactivation, and drug efflux; acquired resistance mechanisms used may be drug target modification, drug inactivation, and drug efflux. Because of differences in structure, etc., there is variation in the types of mechanisms used by gram negative bacteria versus gram positive bacteria. Gram negative bacteria make use of all four main mechanisms, whereas gram positive bacteria less commonly use limiting the uptake of a drug (don't have an LPS outer membrane), and don't have the capacity for certain types of drug efflux mechanisms.

1. Limiting drug uptake

As already mentioned, there is a natural difference in the ability of bacteria to limit the uptake of antimicrobial agents. The structure and functions of the LPS layer in gram negative bacteria provides a barrier to certain types of molecules. This gives those bacteria innate resistance to certain groups of large antimicrobial agents . The mycobacteria have an outer membrane that has a high lipid content, and so hydrophobic drugs such as rifampicin and the fluoroquinolones have an easier access to the cell, but hydrophilic drugs have limited access .

Bacteria that lack a cell wall, such as Mycoplasma and related species, are therefore intrinsically resistant to all drugs that target the cell wall including β-lactams and glycopeptides . Gram positive bacteria do not possess an outer membrane, and restricting drug access is not as prevalent. In the enterococci, the fact that polar molecules have difficulty penetrating the cell wall gives intrinsic resistance to aminoglycosides. Another gram positive bacteria, Staphylococcus aureus, recently has developed resistance to vancomycin. Of the two mechanisms that S. aureus uses against vancomycin, a yet unexplained mechanism allows the bacteria to produce a thickened cell wall which makes it difficult for the drug to enter the cell, and provides an intermediate resistance to vancomycin. These strains are designated as VISA strains .

In those bacteria with large outer membranes, substances often enter the cell through porin channels. The porin channels in gram negative bacteria generally allow access to hydrophilic molecules . There are two main ways in which porin changes can limit drug uptake: a decrease in the number of porins present, and mutations that change the selectivity of the porin channel ]. Members of the Enterobacteriaceae are known to become resistant due to reducing the number of porins (and sometime stopping production entirely of certain porins). As a group, these bacteria reduce porin number as a mechanism for resistance to carbapenems . Mutations that cause changes within the porin channel have been seen in E. aerogenes which then become resistant to imipenem and certain cephalosporins, and in Neisseria gonorrhoeae which then become resistant to β-lactams and tetracycline.

Another widely seen phenomenon in bacterial colonization is the formation of a biofilm by a bacterial community. These biofilms may contain a predominant organism (such as by Pseudomonas aeruginosa in the lung), or may consist of a wide variety of organisms, as seen in the biofilm community of normal flora in the gut. For pathogenic organisms, formation of a biofilm protects the bacteria from attack by the host immune system, plus provides protection from antimicrobial agents. The thick, sticky consistency of the biofilm matrix which contains polysaccharides, and proteins and DNA from the resident bacteria, makes it difficult for antimicrobial agents to reach the bacteria. Thus, to be effective, much higher concentrations of the drugs are necessary. In addition the bacterial cells in the biofilm tend to be sessile (slow metabolism rate, slow cell division), so antimicrobials that target growing, dividing bacterial cells have little effect. An important observation about biofilms is that it is likely that horizontal transfer of genes is facilitated by the proximity of the bacterial cells. That means that sharing of antimicrobial resistance genes is potentially easier for these bacterial communities.

### Modification of drug targets

There are multiple components in the bacterial cell that may be targets of antimicrobial agents; and there are just as many targets that may be modified by the bacteria to enable resistance to those drugs. One mechanism of resistance to the β-lactam drugs used almost exclusively by gram positive bacteria is via alterations in the structure and/or number of PBPs (penicillin-binding proteins). PBPs are transpeptidases involved in the construction of peptidoglycan in the cell wall. A change in the number (increase in PBPs that have a decrease in drug binding ability, or decrease in PBPs with normal drug binding) of PBPs impacts the amount of drug that can bind to that target. A change in structure (e.g. PBP2a in S. aureus by acquisition of the mecA gene) may decrease the ability of the drug to bind, or totally inhibit drug binding

The glycopeptides (e.g. vancomycin) also work by inhibiting cell wall synthesis, and lipopeptides (e.g. daptomycin) work by depolarizing the cell membrane. Gram negative bacteria (thick LPS layer) have intrinsic resistance to these drugs . Resistance to vancomycin has become a major issue in the enterococci (VRE—vancomycin-resistant enterococci) and in Staphylococcus aureus (MRSA). Resistance is mediated through acquisition of van genes which results in changes in the structure of peptidoglycan precursors that cause a decrease in the binding ability of vancomycin . Daptomycin requires the presence of calcium for binding. Mutations in genes (e.g. mprF) change the charge of the cell membrane surface to positive, inhibiting the binding of calcium, and therefore, daptomycin

Resistance to drugs that target the ribosomal subunits may occur via ribosomal mutation (aminoglycosides, oxazolidinones), ribosomal subunit methylation (aminoglycosides, macrolides—gram positive bacteria, oxazolidinones, streptogramins) most commonly involving erm genes, or ribosomal protection (tetracyclines). These mechanisms interfere with the ability of the drug to bind to the ribosome. The level of drug interference varies greatly among these mechanisms

For drugs that target nucleic acid synthesis (fluoroquinolones), resistance is via modifications in DNA gyrase (gram negative bacteria—e.g. gyrA) or topoisomerase IV (gram positive bacteria—e.g. grlA). These mutations cause changes in the structure of gyrase and topoisomerase which decrease or eliminate the ability of the drug to bind to these components

For the drugs that inhibit metabolic pathways, resistance is via mutations in enzymes (DHPS—dihydropteroate synthase, DHFR—dihydrofolate reductase) involved in the folate biosynthesis pathway and/or overproduction of resistant DHPS and DHFR enzymes (sulfonamides—DHPS, trimethoprim—DHFR). The sulfonamides and trimethoprim bind to their respective enzymes due to their being structural analogs of the natural substrates (sulfonamides—p-amino-benzoic acid, trimethoprim—dihydrofolate). The action of these drugs is through competitive inhibition by binding in the active site of the enzymes. Mutations in these enzymes are most often located in or near the active site, and resulting structural changes in the enzyme interfere with drug binding while still allowing the natural substrate to bind.

### Drug inactivation

There are two main ways in which bacteria inactivate drugs; by actual degradation of the drug, or by transfer of a chemical group to the drug. The β-lactamases are a very large group of drug hydrolyzing enzymes. Another drug that can be inactivated by hydrolyzation is tetracycline, via the tetX gene.

Drug inactivation by transfer of a chemical group to the drug most commonly uses transfer of acetyl, phosphoryl, and adenyl groups. There are a large number of transferases that have been identified. Acetylation is the most diversely used mechanism, and is known to be used against the aminoglycosides, chloramphenicol, the streptogramins, and the fluoroquinolones. Phosphorylation and adenylation are known to be used primarily against the aminoglycosides.

### Drug efflux

Bacteria possess chromosomally encoded genes for efflux pumps. Some are expressed constitutively, and others are induced or overexpressed (high-level resistance is usually via a mutation that modifies the transport channel) under certain environmental stimuli or when a suitable substrate is present. The efflux pumps function primarily to rid the bacterial cell of toxic substances, and many of these pumps will transport a large variety of compounds (multi-drug [MDR] efflux pumps). The resistance capability of many of these pumps is influenced by what carbon source is available .

Most bacteria possess many different types of efflux pumps. There are five main families of efflux pumps in bacteria classified based on structure and energy source: the ATP-binding cassette (ABC) family, the multidrug and toxic compound extrusion (MATE) family, the small multidrug resistance (SMR) family, the major facilitator superfamily (MFS), and the resistance-nodulation-cell division (RND) family. Most of these efflux pump families are single-component pumps which transport substrates across the cytoplasmic membrane. The RND family are multi-component pumps (found almost exclusively in gram negative bacteria) that function in association with a periplasmic membrane fusion protein (MFP) and an outer membrane protein (OMP-porin) to efflux substrate across the entire cell envelope.

There are instances where other efflux family members act with other cellular components as multicomponent pumps in gram negative bacteria. One member of the ABC family, MacB, works as a tripartite pump (MacAB-TolC) to extrude macrolide drugs. A member of the MFS, EmrB, works as a tripartite pump (EmrAB-TolC) to extrude nalidixic acid in E. coli.

Efflux pumps found in gram positive bacteria may confer intrinsic resistance because of being encoded on the chromosome. These pumps include members of the MATE and MFS families and efflux fluoroquinolones. There are also gram positive efflux pumps known to be carried on plasmids. Currently, the characterized pumps in gram positive bacteria are from the MFS family . Efflux pumps found in gram negative bacteria are widely distributed and may come from all five of the families, with the most clinically significant pumps belonging to the RND family.