Bacterial Biochemistry

Mechanism of Resistance

There are four major mechanisms that mediate bacterial resistance to drugs .

(1)Bacteria produce enzymes that inactivate the drug; eg, lactamases can inactivate penicillins and cephalosporins by cleaving the lactam ring of the drug.

(2) Bacteria synthesize **modified targets** against which the drug has no effect; eg, a mutant protein in the 30S ribosomal subunit can result in resistance to streptomycin, and a methylated 23S rRNA can result in resistance to erythromycin.

(3) Bacteria decrease their **permeability** such that an effective intracellular concentration of the drug is not achieved; eg, changes in porins [membrane transport proteins] can reduce the amount of penicillin entering the bacterium.

(4) Bacteria actively export drugs using a "multidrug resistance pump" (MDR pump, or "efflux" pump).

The MDR pump imports protons and, in an exchange-type reaction, exports a variety of foreign molecules including certain antibiotics, such as quinolones.

Mechanism	Important Example	Drugs Commonly Affected
Inactivate drug	Cleavage by β-lactamase	β-Lactam drugs such as penicillins, cephalosporins
Modify drug target in bacteria	1. Mutation in penicillin-binding proteins	Penicillins
	2. Mutation in protein in 30S ribosomal subunit	Aminoglycosides, such as streptomycin
	3. Replace alanine with lactate in peptidoglycan	Vancomycin
	4. Mutation in DNA gyrase	Quinolones
	5. Mutation in RNA polymerase	Rifampin
	6. Mutation in catalase-peroxidase	Isoniazid
Reduce permeability of drug	Mutation in porin proteins	Penicillins, aminoglycosides, and others
Export of drug from bacteria	Multidrug resistance pump	Tetracyclines, sulfonamides

GENETIC BASIS OF RESISTANCE

- Chromosome-Mediated Resistance
- Plasmid-Mediated Resistance
- Transposon-Mediated Resistance

Chromosome-Mediated Resistance

- Chromosomal resistance is due to a mutation in the gene that codes for either the target of the drug or the transport system in the membrane that controls the uptake of the drug.

- The frequency of spontaneous mutations usually ranges from 10^{-7} to 10^{-9}

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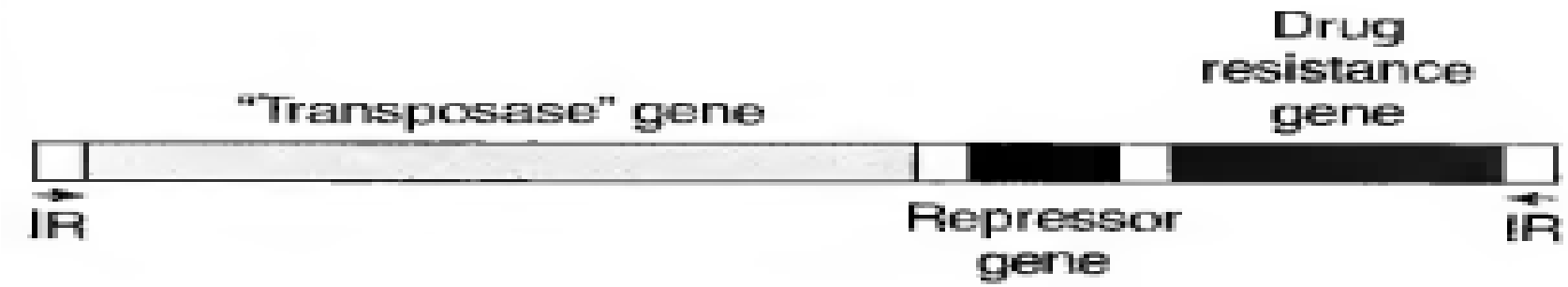
1. *If the frequency that a bacterium mutates to become resistant to antibiotic A (1 in 10 million) and the frequency that the same bacterium mutates to become resistant to antibiotic B is (1 in 100 million)*
2. *the chance that the bacterium will become resistant to both antibiotics (assuming that the antibiotics act by different mechanisms) is the* ***product of the two probabilities****, or 10 15.*
3. *It is therefore highly unlikely that the bacterium will become resistant to both antibiotics.*

4. *Stated another way, although an organism may be resistant to one antibiotic, it is likely that it will be effectively treated by the other antibiotic.*

- R factors may carry one antibiotic resistance gene or may carry two or more of these genes.
- The medical implications of a plasmid carrying more than one resistance gene is 2-fold:
- first and most obvious is that a bacterium containing that plasmid can be resistant to more than one class of antibiotics (eg, penicillin's and amino- glycosides)
- second, that the use of an antibiotic that selects for an organism resistant to one antibiotic will select for an organism that is resistant to all the antibiotics whose resistance genes are carried by the plasmid.

Transposon-Mediated Resistance

- Transposons are genes that are transferred either within or between larger pieces of DNA such as the bacterial chromosome and plasmids.



Type of Bacteria	Clinically Significant Drug Resistance
Gram-positive cocci	
Staphylococcus aureus	Penicillin G, nafcillin
Streptococcus pneumoniae	Penicillin G
Enterococcus faecalis	Penicillin G, aminoglycosides, vancomycin
Gram-negative cocci	
Neisseria gonorrhoeae	Penicillin G
Gram-positive rods	
None	
Gram-negative rods	
Haemophilus influenzae	Ampicillin
Pseudomonas aeruginosa	β-Lactams,[1] aminoglycosides
Enterobacteriaceae[2]	β-Lactams,[1] aminoglycosides
Mycobacteria	
M. tuberculosis[3]	Isoniazid, rifampin
M. avium-intracellulare	Isoniazid, rifampin, and many others

SPECIFIC MECHANISMS OF RESISTANCE

- Penicillins & Cephalosporins. Cleavage by lactamases
- produced by various organisms have different properties.
- For example, **staphylococcal penicillinase** is inducible by penicillin and is secreted into the medium.
- In contrast, some lactamases produced by several gram-negative rods are constitutively produced, are located in the **periplasmic space** near the peptidoglycan, and are **not** secreted into the medium.
- . Clavulanic acid and sulbactam are penicillin analogues that bind strongly to lactamases and inactivate them.
- Combinations of these inhibitors and penicillins, eg, davu- lanic acid and amoxicillin (Augmentin), can overcome resistance mediated by many but not all lactamases.

Vancomycin

- Resistance to vancomycin is caused by a change in the peptide component of **peptidoglycan** from D-alanyl-D-alanine, which is the normal binding site for vancomycin, to D-alanine-D-lactate, to which the drug does not bind.
- Of the four gene loci mediaing vancomycin resistance, VanA is the most important.
- It is carried by a transposon on a plasmid and provides high-level resistance to both vancomycin and teichoplanin.
- The VanA locus encodes the enzymes that synthesize D-ala-D-lactate as well as several regulatory proteins.
- Rare isolates of S. aureus that exhibit resistance to vancomycin

Aminoglycosides

- Resistance to aminoglycosides occurs by three mechanisms:
- (1) modification of the drugs by plasmid-encoded phosphorylating, adenylylating, and acetylating enzymes
- (2) chromosomal mutation, eg, a mutation in the gene that codes for the target protein in the 30S subunit of the bacterial ribosome
- (3) decreased permeability of the bacterium to the drug.

Tetracyclines

- Resistance to tetracyclines is the result of failure of the drug to reach an inhibitory concentration inside the bacteria.
- This is due to plasmid-encoded processes that either reduce uptake of the drug or enhance its transport out of the call.

Chloramphenicol

Resistance to chloramphenicol is due to a plasmid-encoded acetyltransferase that acetylates the drug, thus inactivating it.

Erythromycin

Resistance to erythromycin is due primarily to a plasmid-encoded enzyme that methylate the 23S rRNA, thereby blocking binding of the drug. An efflux pump that reduces the concentration of erythromycin within the bacterium causes low-level resistance to the drug.

- Sulfonamides. Resistance to sulfonamides is mediated primarily by two mechanisms:
- (1) a plasmid-encoded transport system that actively exports the drug out of the cell; and
- (2) a chromosomal mutation in the gene coding for the target enzyme dihydropteroate synthetase, which reduces the binding affinity of the drug.
- Trimethoprim. Resistance to trimethoprim is due primarily to mutations in the chromosomal gene that encodes dihydrofolate reductase, the enzyme that reduces dihydrofolate to tetrahydrofolate.
- Quinolones. Resistance to quinolones is due primarily to chromosomal mutations that modify the bacterial DNA gyrase.
- Resistance can also be caused by changes in bacterial outer-membrane proteins that re- sult in reduced uptake of drug into the bacteria.

- Rifampin. Resistance to rifampin is due to a chro-mosomal mutation in the gene for the subunit of the bacterial RNA polymerase, resulting in ineffective binding of the drug.
- Because resistance occurs at high frequency .rifampin is not prescribed alone for the treatment of infections.
- It is used alone for the prevention of certain infections because it is administered for only a short time
- Isoniazid. Resistance of Mycobacterium tuberculosis to isoniazid is due to mutations in the organism's cata-lase-peroxidase gene.
- Catalase or peroxidase enzyme activity is required to synthesize the metabolite of isoniazid that actually inhibits the growth of M. tuberculosis.

- Ethambutol. Resistance of M. tuberculosis to ethambutol is due to mutations in the gene that encodes arabinosyl transferase, the enzyme that synthesizes the arabinogalactan in the organism's cell wall.
- Pyrazinamide. Resistance of M. tuberculosis to pyrazinamide (PZA) is due to mutations in the gene that encodes bacterial amidase, the enzyme that converts PZA to the active form of the drug, pyrazinoic add.

Drug	Mechanism of Resistance
Penicillins and cephalosporins	β-Lactamase cleavage of β-lactam ring
Aminoglycosides	Modification by acetylation, adenylylation, or phosphorylation
Chloramphenicol	Modification by acetylation
Erythromycin	Change in receptor by methylation of rRNA
Tetracycline	Reduced uptake or increased export
Sulfonamides	Active export out of the cell and reduced affinity of enzyme

NONGENETIC BASIS OF RESISTANCE

- Bacteria can be walled off within an abscess cavity that the **drug cannot penetrate effectively**. Surgical drainage is therefore a necessary adjunct to chemotherapy.
- Bacteria can be in a resting state, ie, not growing; they are therefore insensitive to cell wall inhibitors such as penicillins and cephalosporins.

- Organisms that would ordinarily be killed by penicillin can lose their cell walls, survive as **protoplasts**, and be insensitive to cell-wall-active drugs.

- The presence of foreign bodies makes successful antibiotic treatment more difficult [catheters]

- Failure of the patient to take the drug (noncompliance, nonadherence) is another artifact.

SELECTION OF RESISTANT BACTERIA BY OVERUSE & MISUSE OF ANTIBIOTICS

- prescribe unnecessarily long courses of antibiotic therapy
- sold over the counter to the general public
- Antibiotics are used in animal feed to prevent infections and promote growth

Antagonism

- the combination of a penicillin and an aminoglycoside such as gentamicin has a synergistic action against enterococci because penicillin damages the cell wall sufficed to enhance the entry of aminoglycoside.
- When given alone, neither drug is effective.

antagonism

- Although antagonism between two antibiotics is unusual, one example is clinically important.
- This involves the use of penicillin G combined with the bacteriostatic drug tetracycline in the treatment of meningitis caused by S. pneumoniae.
- Antagonism occurs because the tetracycline inhibits the growth of the organism, thereby preventing the bactericidal effect of penicillin G, which kills growing organisms only.