MECHANISMS OF ANTIBIOTIC RESISTANCE

- By:
- Prof. Dr. Rafal K. Farhan

MECHANISMS OF RESISTANCE

- Enzymatic inhibition
- Alteration of bacterial membranes
  - Outer membrane permeability
  - Inner membrane permeability

- Rapid ejection of the drug [efflux] or reduced drug influx.
- Bypass of antibiotic inhibition.
- Alteration of target sites
  - Altered ribosomal target sites
  - Altered cell wall precursor targets
  - Altered target enzymes

Molecular genetics of antibiotic resistance

- Genetic variability is essential for microbial evolution. It may occur in a variety of ways :-
  - Micro evolutionary changes by point mutations-in nucleotide base pair
  - Micro evolutionary changes [whole scale changes] like

  -Inversions
  - Duplications
  - Deletions
  - Transposition
  - Acquisition of foreign Dna –plasmid mediated/bacteriophages/transposons

1.Enzymatic inhibition

Enzymes inactivating antibiotics

- Beta-lactamases-split amide bond of the beta lactam ring.
- There are many types-characterised by amino acid and nucleotide sequencing
  - ✓ Class A MWT 29000-Preferentially hydrolyze penicillins e.g. TEM-1 prevalent in many gram neg
  - ✓ Class B-metalloenzymes have a zinc –binding thiol group required for beta lactamase activity
  - ✓ Class C mwt 39000 –mainly cephalosporinases
  - ✓ Class D-oxacillin –hydrolyzing enzymes

  Many beta lactamases are plasmid mediated all are produced constitutively there are 6 main groups

  1. Those that hydrolyze benzylpenicillin

Beta lactamases

1. Those that hydrolyze oxacillin and related penicillins
2. Carbenicillinases
3. Those that break extended spectrum beta lactams like aztreonam
4. Those that break down oxyimino B lactams
5. Carbapenemases-found in pseudomonas

Most cephalosporinases are inhibited by clavulanate,sulbactam or tazobactam. Carbapenamases are metalloenzymes inhibited by EDTA but not clavulanate or sulbactam

Production of enzymes modifying antibiotics

- . Aminoglycosides, chloramphenicol-coded by plasmids or chromosomal genes

Modifying enzymes

- Reactions are-
  - N-acetylation
  - O nuleotidylation
  - O phosphorylation
- Chloramphenicol acetyltransferase-inactivates the drug by 3-o-acetylation-plasmid mediated/chromosomal
- Erythromycin esterase-seen in E coli-hydrolyze lactone ring thus deactivating it-limits utility of oral erythromycin in reducing the aerobic gram neg flora of the GIT prior to Gi surgery.

Alteration of bacterial membranes

- *Outer membrane permeability*—outer membrane of gram neg acts as a barrier to antibiotics esp hydrophobic ones.
- *Inner* membrane *permeability-* rate of entry of aminoglycosides into bacterial cells is a function of them binding to a non saturable anionic transporter,where they retain their positive charge and are pulled across the cytoplasmic membrane by the internal charge of the cell.This is an energy dependent process. The energy generation or proton motive force may be altered through mutation

Alteration of bacterial membranes continued

- Promotion of antibiotic efflux-major mechanism for tetracycline resistance in gram neg-plasmid/chromosomal/transposone mediated

.Efflux /influx mechanism

- Bacterial cells have an intrinsic capacity to restrict the entry of small molecules especially gram neg-outer membrane is protective,gram pos no outer membrane hence more antibiotic sensitive
- Restriction of influx is a physiological way to reduce toxixity to bacterial cell.

3. Modification of target sites

- Alteration of ribosomal target sites-hence failure to inhibit protein synthesis and cell growth.
- Affected antibiotics are aminoglycosides ,tetracylines,macrolides,lincosamides.

Altered cell wall precursor targets

- Glycopeptides like vancomycin-bind D-alanine-D-alanine which is present at the termini of peptidoglycan precursors.
- The large glycopeptide molecules prevent the incorporation of the pre cursors into the cell wall

Alteration of target enzymes

- Alteration of PBPs in B lactams
- SMX/TMP-production of a dihdropteroate synthetase that is resistant to binding by sulphonamides-plasmid mediated
- Quinolones-DNA gyrase is made up of gyr Aand gyr B genes-mutations in gyr A result in resistance

By pass inhibition

- Development of auxotrophs-have growth factor requirements different from those of wild strain these mutants require subtrates that normally are synthesized by the target enzymes and if these are present in the environment the organisms grow despite inhibition by synthetic enzymes

Factors promoting drug resistance

- Exposure to sub-optimal levels of antimicrobial
- Exposure to microbes carrying resistance genes
- Inappropriate drug use-

✓ Lack of quality control in manufacture or outdated antimicrobial

✓ Inadequate surveillance or defective susceptibility assays

✓ Poverty or war

✓ Use of antibiotics in foods-Antibiotics are used in animal feeds and sprayed on plants to prevent infection and promote growth Multi drug-resistant *Salmonella typhi has been found in 4 states in* 18 people who ate beef fed antibiotics

Antibiotics and mechanisms of resistance

ANTIBIOTIC	TARGET	MOA	MECHANISM OF RESISTANCE	
CELL WALL				
b-Lactams	Transpeptidases/transglycosylases (PBPs	Blockade of cross linking enzymes in peptidoglycan layer	b-Lactamases, PBP mutants	
Vancomycin	D-Ala-D-Ala termini of peptidoglycan and of lipid II	Sequestration of substrate required for cross linking	Reprogramming of D-Ala-D-Ala to D-Ala-D-Lac od D-Ala –D-ser	
PROTEIN				