Antimicrobials

- Among the most widely used drugs
- Annual expenditure worldwide > U$20 Billion
- Represents 20-40% of drugs administered in hospitals
- Used singly or in combinations
- Choice – should be individualized for each patient
- Specific for a particular infectious agent (if known)
- In hospitals – preferably in consonant with results of antimicrobial sensitivity tests
- If aetiology unknown – usually broad-spectrum, but to be judiciously used!

Bacterial Resistance to Antimicrobials

Antibiotics/Antimicrobials – Wonder drug? Magic Bullets?
Perception eroded – emergence of bacterial resistance to antimicrobials
Timeline illustrating the emergence of antimicrobial resistance in Gram positive cocci:

- 1950's – Penicillin resistant Staph. Aureus
- 1980's – Methicillin resistant S. Aureus (MRSA)
- 1980's – Introduction of Vancomycin
- 1990's – Vancomycin resistant Enterococcus (VRE)
- 1997 – Vancomycin (glycopeptides) – intermediate
- 2002 – Vancomycin resistant S. Aureus

Mechanisms of Resistance

1. Accumulation Barriers to an antimicrobial due to impermeability or active efflux
2. Alteration of antimicrobic targets which render it insusceptible
3. Inactivation of an antimicrobial by an enzyme produced by the microorganism

1. Accumulation Barriers to an antimicrobial due to impermeability of active efflux

- Cell wall (particularly; the outer membrane of Gram negative bacteria) = A formidable barrier to the interior of a cell
- Porins – Outer membrane protein channels that allow antimicrobial penetration depending on the antimicrobial agent's:
- Major reasons for INHERENT resistance to Antimicrobials:

1. Size
2. Charge
3. Degree of hydrophobicity
4. General molecular configuration

- BUT these transport characteristics may change due to **MUTATIONS** in porin proteins.
  e.g; *Pseudomonas Aeruginosa resistance to Imipenem* due to loss of normal porin (Mutation).
- Some Antimicrobials must be actively transported into the cell.
- Some bacteria lack necessary oxidative pathways for transport of aminoglycosides.
  e.g; Streptococci, Enterococci & Anaerobes – R
- Some bacteria have energy dependent EFFLUX systems to pump tetracyclines or quinolones OUT of the cell.

2. Alteration of antimicrobic targets which render it insusceptible

- In bacterial cells – Antimicrobials act by binding and inactivating a target.

- This target is usually a:
  a. Crucial enzyme
  b. Ribosomal Site

- If the target is altered:
  - Reduced affinity for antimicrobials
  - Reduced inhibitory effect of antimicrobials

- Even substitution of 1 Amino Acid at a certain location in a protein can reduce affinity to antimicrobials. This mutation can even occur during therapy!
  Examples:
  - Streptomycin -> To single ribosomal site
  - Nalidixic Acid -> To only 1 of 4 topoisomerase subunits!

- Newer Antimicrobials bind at **multiple sites**, so resistance due to mutation is **very unlikely**.

<u>B-Lactam family and their target site</u>

- Peptidoglycan Transpeptidase Penicillin-Binding Proteins (PBP's)
- **Changes** in 1 or more of these PBP's results in **reduced susceptibility to multiple β-Lactams!**
- These **changes** could be due to:
  - Point mutation
  - Substitutions of amino acid sequences
  - Even synthesis of new enzymes

Reduction in susceptibility due to PBP alterations:

- May be small & incremental (can increase dosage) or may be associated with treatment failures (even if dosage is increased)
- Affect all β-Lactams!
- Prime reason for emergence or MRSA!
- Is one of MULTIPLE mechanisms of resistance like other bacteria such as Enterococci, Gonococci, H.Influenzae and other Gram negative/positive spp.
- New enzymes produced in Vancomycin resistant enterococci leads to:
  - Resistance to sulphonamides, trimethroprim
  - Resistance to clindamycin, erythromycin & other macrolides
  - Enzyme methylates rRNA --> Attachment to mRNA

<u>3. Inactivation of an antimicrobial by an enzyme produced by the microorganism</u>

- Most powerful and most robust of the resistance mechanisms
- Many distinct enzymes can inactivate antimicrobials:
  - In the cell
  - In the periplasmic space
  - Outside the cell
- Act on antimicrobial molecule by
  - Disrupting its structure
  - Catalyzing a reaction that chemically modifies it

β-Lactamases

- A general term referring to any of >100's of bacterial enzymes, capable of breaking open the β-Lactam ring --> Inactivate various members of the β-Lactam group

- First discovered – S. Aureus inactivated penicillin in vitro. The enzyme was then called penicillinase

- More such enzymes then discovered
- Grouped as a family of β-Lactamases, each distinct with own properties and substrates.

- Example: Staph Penicillinase also acts on ampicillin BUT NOT ON Methicillin or cephalosporins

- To keep track of β-Lactamase identifiers, Classifications/Schemes were created based on:
  - Molecular structure
  - Substrate Profile
  - Inducibility (produced constitutively or needs induction)
  Examples: TEM-1, TEM-2, OXA, SVH, etc.

Gram Positive β-Lactamases

- Are coenzymes
- Not active against cephalosporins, nor against methicillin & oxacillin
- Bound by β-Lactamase inhibitors like clavulanic acid

Gram Negative β-Lactamases

- Act in periplasmic spaces
- May have penicillinase and/or cephalosporinase activity
- May or may not be inhibited by clavulanic acid
- Constitutively produced at very low levels BUT inducible to higher levels by β-Lactam agent exposure
- NEW class: ESBL's (Extended-spectrum β-Lactames) = VERY WORRISOME!
- β-Lactamase-producing bacteria usually demonstrate high level resistance. Even weak producers are considered resistant.
- Rapid distinct tests for β-Lactamase are available.

Modifying Enzymes

The most common cause of acquired bacterial resistance to aminoglycosides is through production of >50 enzymes that ACETYLATE, ADENYLATE, PHOSPHORYLATE and ….(tak sempat salin)

Genetics of Antimicrobial Resistance

- Intrinsic/Chromosomally Resistant Bacteria:
  Inherent resistance:-
  a. Permeability barriers
  b. Cell wall not susceptible
  c. Chromosomal genes encode β-Lactamases, etc

  Acquired resistance:-
  a. Mutational (Single or Multiple)
  b. Plasmids & Conjugation
  c. Transposons & Transpositrons
  d. Transformation
  e. Transduction

Control of Bacterial Resistance (General Principles)

The Antimicrobial is to be:

- Used conservatively & specifically in therapy

- Used in adequate doses and duration
- Selected according to proven and anticipated susceptibility of strain
- Use narrow rather than broad spectrum when specific infections know
- Used in combination if emergence of resistance is anticipated
- Used as prophylaxis only when necessary and in short duration

Other measures to prevent cross infections

- Rigid aseptic procedures
- Handwashing
- Containment/Isolation of patients
- Protective precautions
- Monitor resistant organisms
- Restrict use of therapeutically valuable antimicrobials

Extra

Physicians: Surgeons don't know anything, but they do everything.
Surgeons: Physicians think they know everything, but they do nothing!
~ Story from Pak Nasa