CHAPTER (7)

ANTIMICROBIAL CHEMOTHERAPY

Definitions:

Antibiotics:

- **Low-molecular weights antimicrobial substances** that are produced as **secondary metabolites by certain groups of microorganisms →** especially Streptomyces, Bacillus & few moulds (Penicillium & Cephalosporium)
- Although their **original source was microorganism →** some antibiotics are **currently made synthetically → chemical modification** of certain **antibiotics** (to achieve the **desired properties**) is prominent method of **new drug development** (**semi-synthetic antibiotics**)

Antimicrobial chemotherapeutic agents:

Chemically synthesized substances that are used to treat infectious diseases **→** by **killing or inhibiting the growth (or multiplication) of microorganisms**

Bacteriostatic agent:

Antimicrobial agent that is capable of **inhibiting bacterial multiplication → multiplication resumes upon removal of the agent**

Bactericidal agent:

Antimicrobial agent that is **capable of killing bacteria → multiplication can not be resumed**

Selective toxicity:

- Ability of **antimicrobial agent to harm pathogen without harming the host**
- **It may be due to:**
  - ❶ Specific **receptor (or target) for the drug** found **in microbe** but **not in human body** (e.g. peptidoglycan)
  - ❷ **Inhibition of biochemical event essential for the organism** but **not for the host**

Spectrum of activity:

Range of microorganisms that are **affected by certain antibiotic** is expressed as its spectrum of action

① Broad spectrum antibiotics	② Narrow spectrum antibiotics	③ Limited spectrum antibiotics
Kill or inhibit the growth of **wide range of Gram-positive & Gram-negative bacteria**	Effective mainly against **either Gram-positive or Gram-negative bacteria**	Effective **against single organism or disease**

Mechanisms of Action of Antimicrobial Agents

① Inhibition of bacterial cell wall synthesis

- These antibiotics are **bactericidal** with **minimal tissue toxicity**
- **Agents acting by this mechanism include:**

❶ β-lactam antibiotics	❷ Glycopeptides	❸ Cycloserine & bacitracin
e.g. penicillins, cephalosporins & others	e.g. vancomycin & teicoplanin	
β-lactam drugs inhibit **last steps of peptidoglycan synthesis** → this inhibition is initiated by **binding of drug to certain cell receptors** known as **penicillin-binding proteins (PBPs)**	On the other hand, Glycopeptides & Cycloserine **inhibit early steps in biosynthesis of peptidoglycan** → which **occur inside the cytoplasmic membrane**	
Therefore, **mechanism of resistance to β-lactam antibiotics** is **different from that for other groups** → So, **vancomycin** could be **used successfully** in **infections caused by β-lactam resistant staphylococci**		

② Interference with the cell membrane function

- These agents are **microbicidal**
- They are **highly toxic** as they **have narrow margin of selective toxicity**
- These agents **disrupt the cytoplasmic membrane** & **interfere with its function** → these include:
  - ❶ **Anti-bacterial agents** → e.g. polymyxin & colistin
  - ❷ **Anti-fungal agents** → e.g. amphotericin B, nystatin & imidazoles

③ Inhibition of bacterial protein synthesis

- Bacteria have **70S ribosomes** (with **30S & 50S subunits**)

Whereas **mammalian cells** have **80S ribosomes** (**40S & 60s subunits**)

→ This difference **makes bacterial ribosomes a selective target for antimicrobials**

- **Agents acting on 30s ribosomal subunit:**
  → e.g. tetracycline & aminoglycosides (gentamicin, amikacin, streptomycin)

- **Agents acting on 50S ribosomal subunit:**
  → e.g. macrolides (erythromycin, azithromycin), lincomycins (clindamycin) , streptogramins, linezolid, chloramphenicol & fusidic acid

④ Inhibition of bacterial nucleic acid synthesis

❶ **Inhibition of RNA synthesis:**
→ through **strong binding to DNA-dependent RNA polymerase** → e.g. **rifampin**

❷ **Inhibition of DNA synthesis:** → through **blocking DNA gyrase** → e.g. **quinolones** & **novobiocin**

❸ **Inhibition of folic acid synthesis:** → by **competitive antagonism** → e.g. **sulphonamides**
- For many organisms, **para-amino benzoic acid (PABA)** is essential for **synthesis of folic acid**
- **Sulphonamides** are **structural analogues of PABA** → they **compete with PABA for active center of the enzyme involved in folic acid synthesis** → as a result, **non-functional analogues** of folic acid are formed and **nucleic acid synthesis is inhibited**

❹ **Inhibition of dihydro-folic acid reductase** leading to **inhibition of active folic acid synthesis** → the latter is important for **purine synthesis** and, consequently, **nucleic acid formation**
→ **examples** of these antimicrobials include **trimethoprim** & **pyrimethamine**

Combined Therapy

- The **ideal rule in antimicrobial therapy** is **mono-therapy** → which means **choosing one drug effective against particular organism**
- However, there are **conditions which necessitate the use of more than one antibiotic** in order to **achieve successful clinical response**

Possible indications:

❶ **Severely ill patients** suspected of **having serious infections** → e.g.:
  ① **Bacterial meningitis**
  ② **Sepsis in immunocomoromised patients** → caused by *Pseudomonas aeruginosa*, *Klebsiella spp.*, *Enterobacter spp.* or *Staph. Aureus*

❷ Febrile neutropaenia

❸ To **delay** the **emergence of drug-resistant mutants** → e.g. in **treatment of T.B.**

❹ To achieve **bactericidal action** through **synergistic effect** → e.g. in **enterococcal endocarditis**

❺ **Mixed infections** → e.g. **infections following massive trauma**

Effects of combined therapy:

① Synergistic effect	② Antagonistic effect	③ Indifference	④ Addition
→ (1 + 1 = > 2)	→ (1 + 1 = < 1)	→ (1 + 1 = 1)	→ (1 + 1 = 2)
The **combined action is significantly greater than** the **sum of both effects**	The **combined action** is **less than** that of the **more effective agent when used alone**	The **combined action** is **no greater than** that of **the more effective agent when used alone**	The **combined action** is **equivalent to the sum** of the **actions of each drug when used alone**
e.g.: ❶ Vancomycin + gentamicin → in treatment of methicillin-resistant staphylococci ❷ Sulfamethoxazole + trimethoprim (cotrimoxazole) → in treatment of shigellosis	**e.g.:** Penicillin + chloramphenicol → in treatment of meningitis	**e.g.:** Cefepime + vancomycin or clindamycin + vancomycin	

Antimicrobial Chemoprophylaxis

- Chemoprophylaxis is **administration of effective antimicrobial agent** → to **prevent rather than to treat** infection with certain microbe → thus **preventing development of a disease**

- **Examples:**

❶	**Preoperative** in some surgical operations	
❷ **Penicillin or erythromycin**	Given to **individuals with abnormal heart valves prior to dental procedures**	To **prevent endocarditis**
❸ **Long acting penicillin** (or **erythromycin**)	Given to **rheumatic patients**	To **prevent re-infection with *S. pyogenes***
❹ **Rifampicin**	Given to **close contacts of meningococcal meningitis** for **2 days**	To **prevent meningitis**

Resistance to Antimicrobial Agents

- Antibiotic resistance is **global problem faced today** in treatment of infectious diseases
- Resistance to antibiotics is **more prevalent in hospitals** → especially **intensive care units** due to higher antibiotic use

- **Resistance to antimicrobial agents is of two categories → either intrinsic or acquired:**

① Intrinsic (inherent or natural) resistance	② Acquired resistance
∗ This type of resistance refers to **bacteria that are insensitive** (in their **natural state**) to antibiotic **without acquisition of resistance factors**	∗ It results from **altered bacterial physiology & structure** due to **changes in genome of organism**
∗ It is **consistent** & can be **expected once the organism is known**	∗ It is **inconsistent** & **unpredictable** → **unpredictable** nature of this **resistance** is the **1ry reason** why **laboratory methods to detect resistance are necessary**
∗ **Intrinsic resistance occurs in the following situations:** ❶ **Streptomycetes** are **protected from the antibiotics they produce** ❷ **Gram -ve cell membrane** has **pores too small to** allow large antibiotic molecules (nafcillin) to penetrate ❸ **Organism lacks target or receptor for antibiotic** → as in case of **resistance of *Enterococcus* species to cephalosporins**	∗ **Acquired resistance mechanisms are driven by two genetic processes in bacteria:** ❶ **Mutation and selection: (sometimes referred to as vertical evolution):** * Exposure of organism to **antibiotic** exerts **selective pressure on organism** & leads to **mutation** * The **more frequent the exposure to antibiotic** → the **greater the potential resistance** ❶ **Exchange of genes between strains & species: (sometimes called horizontal evolution):** Resistance genes can be encoded on plasmids, phages and transposable genetic elements → (see chapter 5)

Mechanisms of acquired resistance: **Bacteria have ability to use one or more of the following mechanisms:**

① Reduction of the intracellular concentration of the antibiotic: → by:

❶ **Decrease in influx of antibiotic:** → through:
① Reduction of permeability of outer membrane by modification
② Loss of porin (hollow membrane protein) required for entry of the antibiotic molecules

❷ **Efflux pumps:**
The antibiotic is **pumped out** across the cytoplasmic membrane **faster than it can diffuse in** → so the concentration of antibiotic remains too low to be effective

② Inactivation of the antibiotic: → e.g.:
❶ Production of **β-lactamases** leads to **hydrolysis of the β-lactam ring** → thus **inactivating penicillins & cephalosporins**
❷ Production of **acetyl transferase** → results in **chloramphenicol resistance**
❸ Production of **aminoglycosides-modifying enzymes**

③ Target modification:

Modification of the target site for the antibiotic → results in reduced affinity for its receptor:

❶ Modification of penicillin-binding proteins (PBPs)	❷ Alteration of 50S ribosomal subunit	❸ Alteration of 30S ribosomal subunit
→ Primary mode of resistance to β-lactam antibiotics in **methicillin-resistant *S. aureus* (MRSA)**	→ Reduces **affinity of macrolides, linezolid & streptogramins for the ribosome**	→ Reduces **affinity of aminoglycosides for the ribosome**

④ Target elimination: → by **developing new metabolic pathways:**

These bacteria have the ability to **create new metabolic pathways** that **bypass the original target** → e.g. **resistance to trimethoprim**

⑤ Target overproduction:

May be mechanism used by ***S. aureus* strains** with **intermediate susceptibility to vancomycin** (**VISA**)

Complications of Chemotherapy

① Toxicity: → may be **dose-dependent or independent** → e.g.:

❶ Chloramphenicol	❷ Aminoglycosides	❸ Tetracycline	❹ Streptomycin
→ Can cause **bone marrow depression**	→ May cause **nephrotoxicity**	→ May cause **staining of teeth in infants**	→ May affect **8th cranial nerve** leading to **vestibular dysfunction**

② Allergy (hypersensitivity): → usually **not dose-dependent** → e.g.:

❶ Penicillins	❷ Local application of sulphonamides
May cause **urticaria**, **anaphylactic shock** or **serum sickness**	May result in **contact dermatitis**

③ Emergence of resistant strains:

- **Abuse of antibiotics** (low dosage, interrupted course, no real indication & improper choice) encourages **emergence of resistant mutants**
- These **mutants will overgrow** and **replace the originally susceptible bacteria**
- It is recommended that **in vitro susceptibility testing should be performed** → to **guide selection of anti-bacterial drugs**

④ Super-infection:

Occurs as a result of **outgrowth of resistant members of normal flora** when **sensitive ones are eradicated during antibiotic therapy** → e.g.:

❶ Oral thrush	❷ Pseudomembranous colitis
Caused by overgrowth of the yeast *Candida*	Caused by overgrowth of *Clostridium difficile*

Choice of Antimicrobial Agent for Therapy

The following are guidelines that can be followed for proper antibiotic use:

❶ Select antibiotic that is able to **penetrate to site of infection** and **achieve effective concentration** → e.g. certain drugs are able to pass **blood-brain barrier**, others are **highly concentrated in urine**

❷ Identify the **nature of the infection** → whether bacterial, viral, fungal, or parasitic
→ (**Common mistake** is to give **anti-bacterial agent for viral infection**)

❸ Choose as **narrow antibiotic spectrum** as you can
→ When you get results of **culture & susceptibility**, revise your treatment to "**narrow-down**" the **spectrum as far as possible**

→ **Use of broad spectrum antibiotics is likely to:**
① Faster induction of **resistance** to antibiotics ② May be complicated by **super-infection**

❹ Give the **appropriate dose** of antibiotic for **proper duration**
→ **inadequate dosage** or **undue prolonged therapy** may result in **drug toxicity & antibiotic resistance**

❺ Know the **potential of the drug to produce toxicity:**
→ Some drugs known to be of **low toxicity** → will exert **high toxicity if they accumulate in blood** due to **liver or kidney dysfunction**

→ Use antibiotics that are **only safe for pregnant and lactating women** and for **infants & children**

❻ Choose **bactericidal rather than bacteriostatic antibiotics**

Microbial Susceptibilities to Antimicrobial Agents

- Microorganisms **vary in their susceptibility to different chemotherapeutic agents**, and susceptibilities can change over time

- Ideally, **appropriate antibiotic to treat** any particular infection should be **determined in vitro before** any **antibiotic is given**

- **In vivo activity** of antimicrobial agent is **not always the same as its in vitro susceptibility** → because it involves **many host factors** that are **not tested in vitro**

- **Activity of antimicrobial agent** against organism is **dependent on its concentration**

- Some idea of the effectiveness of chemotherapeutic agent can be obtained from determining the **minimal inhibitory concentration (MIC):**
  - ∗ MIC is defined as the **lowest concentration of drug that prevents growth of test organism**
  - ∗ The MIC forms the basis for **susceptibility** & **determining breakpoints**

  - ∗ **Breakpoint** of antimicrobial agent → concentration that can be achieved in serum with optimal dose
    - ❶ **Organisms with MICs at or below the breakpoint** → are considered **susceptible**
    - ❷ On the other hand, organisms with **MICs above the breakpoint** → are **considered resistant**

- **Routine in vitro susceptibility testing can be done by one of the following methods:**
  - ❶ Disc diffusion method
  - ❷ Dilution method such as tube broth dilution
  - ❸ Gradient diffusion (E test) method

Empiric Therapy

- The empiric therapy is a **"best guess" procedure** → based upon **provisional diagnosis** made by the physician that a **patient has bacterial infection which requires treatment**
- Depending on **the type of infection**
  → There will be a **short list of bacteria** most likely to be **causing that infection**
  → Depending on **the type of bacteria**
  → There will be **antibiotic most likely to successfully treat** that infection
- "Best guess" treatment is **not always successful** or appropriate → as many **bacteria have unpredictable susceptibilities to antimicrobial agents**

Indications:

❶ **In closed lesions** → where there is **no available sample**

❷ In **seriously ill patients** → empiric therapy should be **started without delay**
→ (but **after collecting specimens for culture**)

Test Yourself

1) Selective toxicity of an antibiotic:

a- Depends on presence of a receptor for the drug in hosts not in organisms
b- Is the ability of the drug to inhibit growth of a wide range of bacteria
c- Depends on inhibition of a biochemical event essential for the host
d- Is the ability of the drug to harm the organism without harming the host
e- Is one of the complications of antibiotic therapy

2) Which of the following antimicrobial agents is most toxic to humans?

a- Bacitracin b- Cephalosporin c- Amphotericin B
d- Penicillin e- Vancomycin

3) One of the following antimicrobial drugs is not among the group acting through inhibition of the bacterial cell wall:

a- Penicillin b- Vancomycin c- Cephalosporins
d- Bacitracin e- Novobiocin

4) Which of the following ABs inhibits bacterial protein synthesis by acting on 30S ribosomal subunit?

a- Vancomycin b- Macrolides c- Polymyxin
d- Aminoglycosides e- Chloramphenicol

5) The following are mechanisms of acquired resistance to antimicrobial agents EXCEPT:

a- Decreasing the influx of the antibiotic b- Modification of t e receptor (target) site
c- Target over rod action d- Absence of cell wall
e- Target elimination by developing new metabolic pathways

6) The MIC is the:

a- Highest concentration of a drug required to inhibit bacterial growth
b- Standard dose a drug required to inhibit bacterial growth
c- Lowest concentration of a drug required to inhibit bacterial growth
d- Lowest dilution the a drug required to inhibit bacterial growth
e- Maximum dose of a drug required to inhibit bacterial growth

7) Combined ant biotic therapy is indicated in the following conditions EXCEPT:

a- Mixed infections b- T.B. c- Viral meningitis
d- Endocarditis e- Febrile neutropenia

8) Regarding the effect of combined therapy with antimicrobial drugs, the expssion (1 + 1 = > 2) means:

a- Antagonistic effect b- Synergistic effect c- Indifference
d- Addition e- Ineffectiveness