Antimicrobial agents

Introduction

- **Antibiotic** -chemical substance produced by various species of microorganisms that is capable in small concentrations of inhibiting the growth of other microorganisms
- **Semi-synthetic antibiotics**- synthetic derivatives of naturally-occurring antibiotics
- **Chemotherapeutic agents**- chemical antimicrobial compounds

Introduction

- **Antimicrobial agent**- chemical substance produced by a microorganism or wholly or partially by chemical synthesis, which in low concentrations, inhibits the growth of other microorganisms
- **Bacteriostatic agents**– antimicrobial agents that inhibit growth and replication of bacteria; are non-lethal
- **Bactericidal agents** – antimicrobial agents that cause bacterial cell death by inhibition of
  - Cell wall synthesis
  - Nucleic acid synthesis
  - Protein synthesis

Introduction

Selective toxicity

- Highly effective against the microbe but have minimal or no toxicity to humans

Classification of antimicrobial agents

1. **Targeted microorganisms**
   - Antibacterial agents
   - Antifungal agents
   - Antiparasitic agents
   - Antimycobacterial agents
2. **Mode of action**

Antibacterial antimicrobial agents

1. Interference with cell wall synthesis
2. Disruption of cell membrane
3. Inhibition of protein synthesis
4. Interference with nucleic acid synthesis

1. Interference with cell wall synthesis

- Act on the formation of the PG layer

1. Beta-lactam antimicrobial agents

- Feature a β-lactam ring in their structure
- Mimic D-Ala:D-Ala moiety
- Form an irreversible intermediate within active site of Penicillin binding proteins (PBPs)
- Weaken Peptidoglycan → subsequent inhibition of cell growth/lysis
  - **Penicillins**
  - **Cephalosporins**
  - **Monobactams**
    - Aztreonam-
      - Bind to PBP3 of aerobic Gram-negative bacteria e.g *Pseudomonas aeruginosa*

1. Interference with cell wall synthesis

- **Carbapenems**-
  - broad spectrum activity
  - Bind to PBP 1 and PBP 2 of gram-negative and positive bacteria
  - Imipenem, Meropenem, Ertapenem
- **Oxa-cephems**-
  - Latamoxef

Beta-lactamase inhibitors

1. Reversible/irreversible binding of the enzyme
   - Form unfavourable steric interactions
   - **Extended-spectrum** cephalosporins, monobactams, Carbapenems
2. Irreversible "suicide inhibitors"
   - Permanently inactivate the β-lactamase
   - **Clavulanic acid**
   - **Sulbactam**
   - **Tazobactam**

* Inhibitor-resistant β-lactamases.

Interference with cell wall synthesis

2. Glycopeptides

- Large molecules unable to penetrate the outer membrane of Gram-negative bacteria
- Spectrum limited to Gram-positive organisms
- Complex with the D-Ala-D-Ala portion of the cell wall precursor
- Last resort for multi-drug resistant Gram-positive organisms e.g MRSA, Enterococci
  - **Vancomycin**
  - **Teicoplanin**
  - **Telavancin**

Interference with cell wall synthesis

3. **Bacitracin**
   - Gram-positive bacteria
   - Too toxic for systemic use
   - Topical; presumptive identification of GAS
4. **Cycloserine**
   - Produced by *Streptomyces*
   - Analogue of D-Alanine
5. **Fosfomycin**
   - Antibiotic produced by *Streptomyces* and *Pseudomonas* spp; broadspectrum,
   - Targets *Mur* enzyme
6. **Isoniazid**

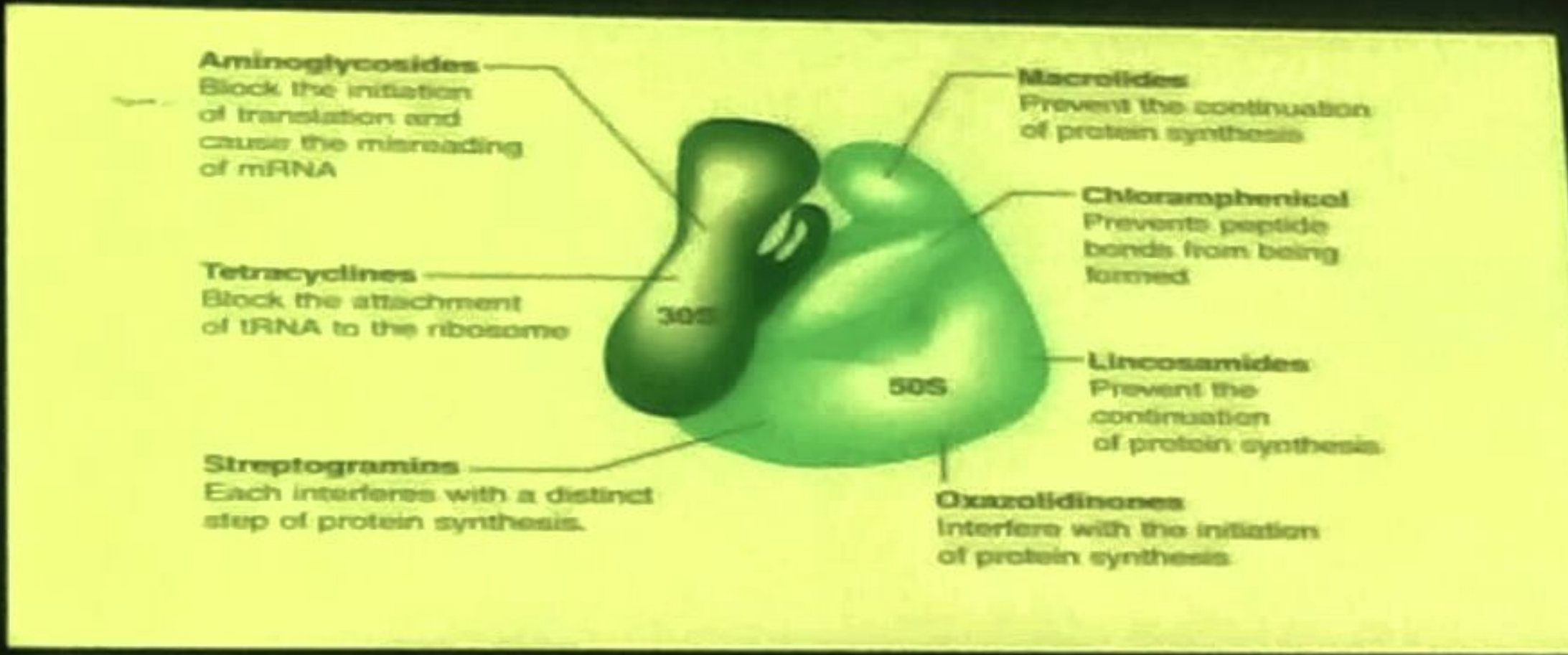
2. Disruption of bacterial membrane structure

- Polymixin B
- Colistin (Polymixin E)
- Derived from *Bacillus polymyxa*
- Act like cationic detergents to disrupt cell membranes
- Limited spectra of activity and significant toxicity (active only against gram-negative rods esp. *Pseudomonas* spp)

3. Inhibition of protein synthesis

30S
Aminoglycosides (Streptomycin, neomycin, kanamycin, gentamicin, amikacin, etc)
Tetracyclines (tetracycline, doxycycline, minocycline, tigecycline)

50S
Macrolides (erythromycin, clarithromycin, azithromycin)
Chloramphenicol, Thiamphenicol
Lincosamides (lincomycin, clindamycin)
Oxazolidinones (linezolid)
Streptogramins (Quinupristin-dalfopristin)


Aminoglycosides
Block the initiation of translation and cause the misreading of mRNA
Tetracyclines
Block the attachment of tRNA to the ribosome
Streptogramins
Each interferes with a distinct step of protein synthesis.
30S
50S
Macrolides
Prevent the continuation of protein synthesis
Chloramphenicol
Prevents peptide bonds from being formed
Lincosamides
Prevent the continuation of protein synthesis
Oxazolidinones
Interfere with the initiation of protein synthesis

4. Interference with nucleic acid synthesi

1. Sulphonamides and diaminopyrimidines

- Affect folic acid metabolism
  - Sulphonamides- analogues of PABA
    - Sulfamethoxazole
    - Sulfadoxine
    - Sulfadiazine
  - Diaminopyrimidines- prevent reduction of DHF to THF
    - Trimethoprim
    - Pyrimethamine
    - Cycloguanil

Interference with nucleic acid synthesis

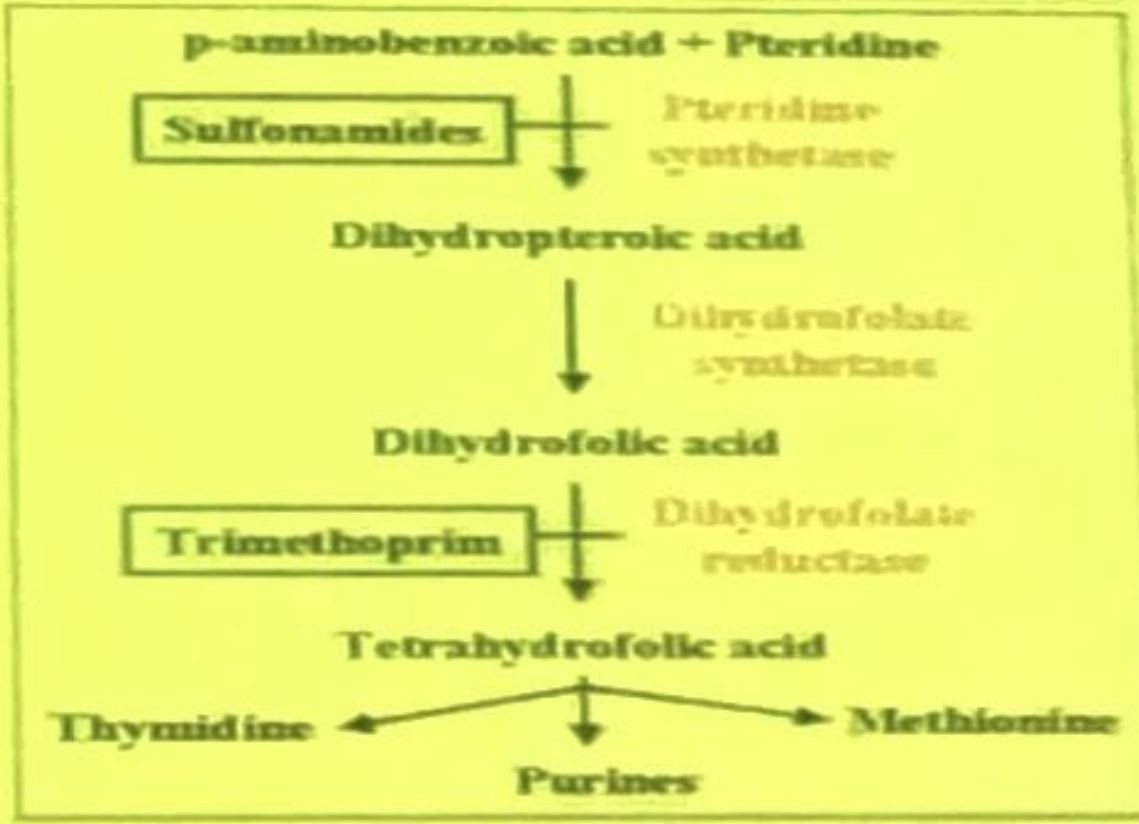
2. **Quinolones**

- Act on α subunit of DNA gyrase
  - Nalidixic acid- active only against Gram-negatives
  - 6-fluoro derivatives
    - Ciprofloxacin
    - Ofloxacin

3. **Nitroimidazoles**

- 5-nitroimidazoles
- Active only against **anaerobic**, certain **microaerophilic** bacteria

Metronidazole Ornidazole
Tinidazole Nimorazole


p-aminobenzoic acid + Pteridine
Sulfonamides
Pteridine synthetase
Dihydropteroic acid
Dihydrofolate synthetase
Dihydrofolic acid
Trimethoprim
Dihydrofolate reductase
Tetrahydrofolic acid
Thymidine
Methionine
Purines

Interference with nucleic acid synthesis

4. **Nitrofurans**
   - Nitrofurantoin
5. **Novobiocin**
   - Acts on β subunit of DNA gyrase
6. **Rifamycins**
   - Inhibit RNA transcription
     - Rifampicin (Rifampin)- antituberculus drug
     - Rifabutin- *M.avium* complex

Antimicrobial combination results

- **Synergism** – the effect of the combination is greater than the sum of the effect of its components penicillin + aminoglycoside
- **Antagonism** – combination of antibiotics in which the activity of one interferes with the activity of the other e.g. a bacteriostatic antibiotic like tetracycline combined with a β lactam antibiotic like penicillin which acts on dividing cells

Antimicrobial resistance

Antimicrobial resistance

- **Definition** – when antimicrobial susceptibility has been lost to such an extent that the drug is no longer effective for use; the organism is said to have achieved resistance

Mechanisms of drug resistance

- **Inherent (natural) resistance**
  - Lack of a transport system for an antimicrobial agent
  - Lack of the target of the antimicrobial molecule
  - Outer membrane that establishes a permeability barrier against the agent — in Gram-negative bacteria

Acquired mechanisms of antimicrobial resistance

- Chromosomal-mediated – Mutation
- Plasmid-mediated – Gene exchange (conjugation in most)

Acquired mechanisms of antimicrobial resistance

1. **Drug inactivation or modification:**
   - Usually plasmid-mediated
   - e.g production of β-lactamases– hydrolyze the β-lactam ring.
     - Penicillins and cephalosporins
   - Enzymes that phosphorylate or adenylate
     - Aminoglycosides
   - Acetylation
     - Chloramphenicol

Acquired mechanisms of antimicrobial resistance

2. Alteration of target site

- Alteration of PBP—the binding target site of penicillins—in MRSA and other penicillin-resistant bacteria
- Mutational changes in 30S ribosomal binding site e.g. resistance of Enterococci to Streptomycin
  - Aminoglycosides – altered protein in 30S ribosome
- Macrolides – methylation of 23S ribosomal RNA, blocking erythromycin binding (plasmid-mediated)

Acquired mechanisms of antimicrobial resistance

3. **Alteration of metabolic pathway:**

- e.g. some sulphonamide-resistant bacteria do not require PABA, Instead, like mammalian cells, they turn to utilizing preformed folic acid.

Acquired mechanisms of antimicrobial resistance

4. **Reduced drug accumulation:**
   - Decreasing drug permeability –
     - change in the no. or character of porin channels e.g. resistance to aminoglycosides, chloramphenicol
   - Increasing active efflux (pumping out using efflux pumps) of the drugs across the cell surface e.g. tetracyclines

Others

- **Overproduction of target enzyme**
  - Sulphonamides – increased levels of dihydropteroate synthetase
  - Trimethoprim – increased levels of DHFR
- **Alteration of intracellular target enzyme**
  - Quinolones – modified DNA gyrase & topoisomerase IV
  - Rifampicin – altered DNA-dependent RNA polymerase

Antimicrobial susceptibility testing(AST)

Antimicrobial susceptibility testing(AST)

Aim of AST

- Select effective antimicrobial drugs
- Detect resistance in individual bacterial isolates

Several methods

1. Qualitative methods
2. Quantitative methods

Antimicrobial susceptibility testing(AST)

1. **Qualitative methods**
   - Disc diffusion (Kirby-Bauer Disk Diffusion Test)
     - Multidiscs
     - Single discs
   - Stoke's method-both test and control organisms are inoculated on the same plate
     - Sensitive organisms : zone of inhibition =/ > that for control organism
     - Resistant organism : zone of inhibition < that for control organism or no zone of inhibition
   - Tablet diffusion
   - Gutter method
   - *Nutrient agar (NA) is used

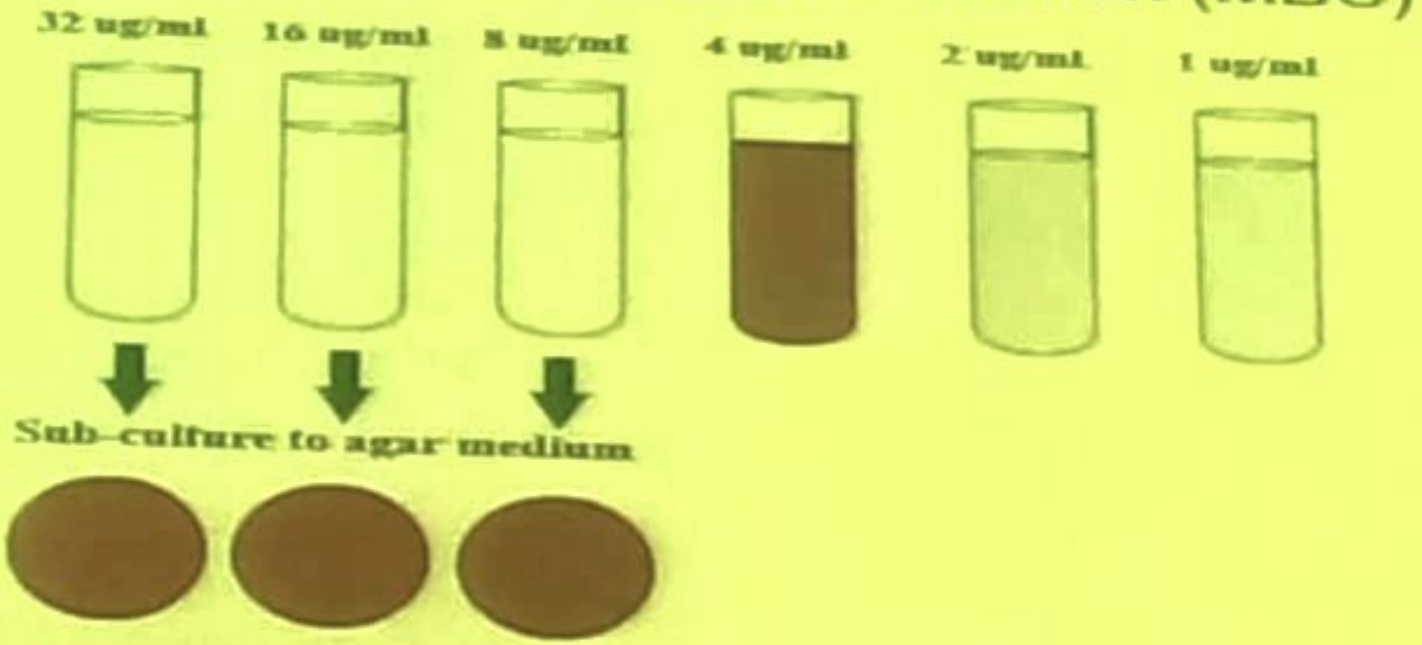
Factors affecting disc diffusion

1. pH of medium
2. Media components
   - Agar depth, nutrients
3. Stability of drug
4. Size of inoculum
5. Length of incubation
6. Metabolic activity of organisms
7. Temperature of incubation

Antimicrobial susceptibility testing

2. **Dilution methods**
   - Tube dilution method
   - Agar dilution method
- Used to measure **MIC** and **MBC**
  - **Minimum inhibitory concentration (MIC)**- lowest concentration of antibiotic that inhibits visible growth
  - **Minimum bactericidal concentration** – lowest concentration of antibiot that kills 99.9% of the inoculum
- Dilutions of an antimicrobial agent are added to a broth or agar medium
- A standardized inoculum is then added (0.5 McFarland)

Minimal Inhibitory Concentration (MIC)
vs.
Minimal Bactericidal Concentration (MBC)

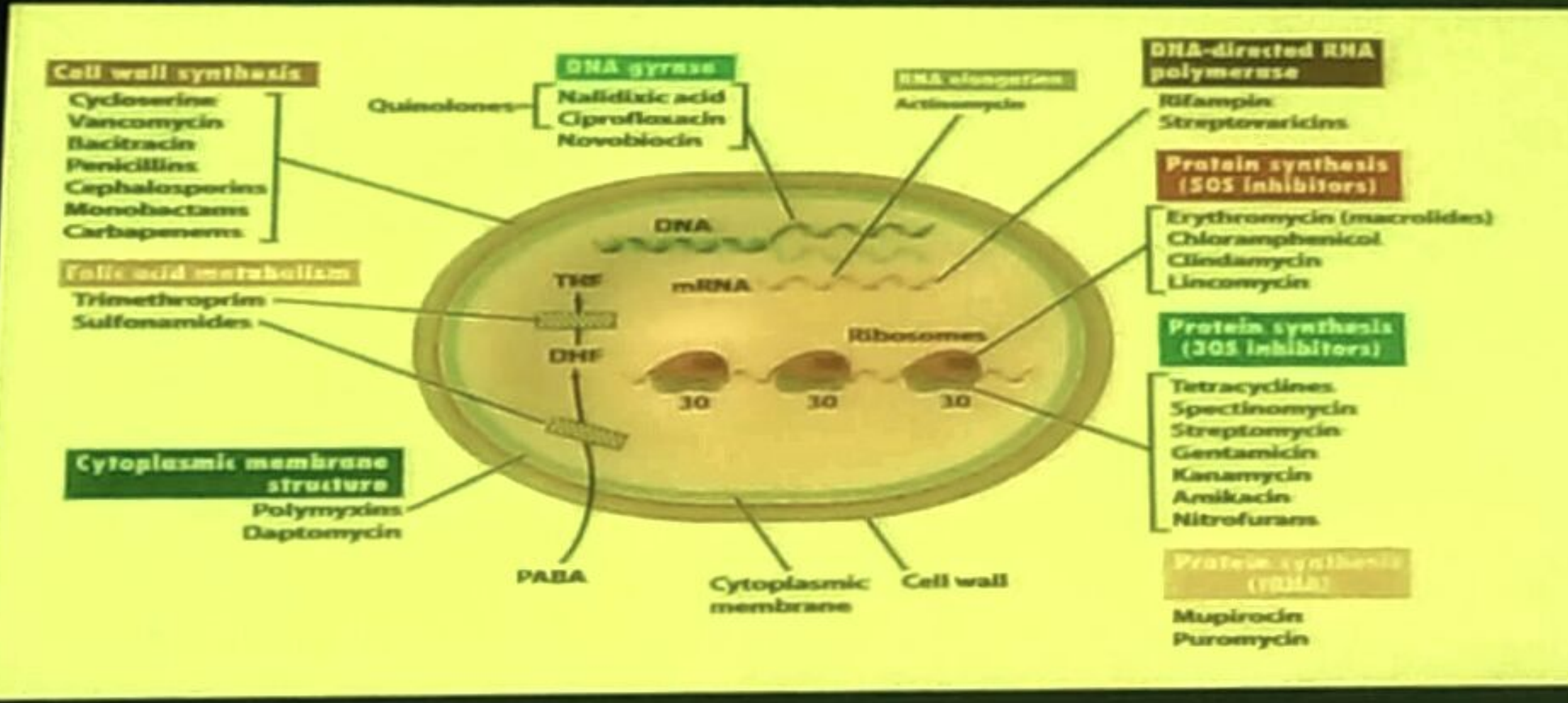


E-Test

- A plastic strip impregnated with antimicrobial agent
- Placed into agar inoculated with bacteria
- Diffusion into the media
- Continuous concentration gradient that yields a quantitative measurement of the MIC value

Mechanism-specific tests

- E.g. Nitrocefin disc for beta-lactamase production. Nitrocefin is a chromogenic cephalosporin


Cell wall synthesis
Cycloserine
Vancomycin
Bacitracin
Penicillins
Cephalosporins
Monobactams
Carbapenems
DNA gyrase
Quinolones
Nalidixic acid
Ciprofloxacin
Novobiocin
RNA elongation
Actinomycin
DNA-directed RNA polymerase
Rifampin
Streptovaricins
Protein synthesis (50S inhibitors)
Erythromycin (macrolides)
Chloramphenicol
Clindamycin
Lincomycin
Folic acid metabolism
Trimethroprim
Sulfonamides
THF
DHF
DNA
mRNA
Ribosomes
30
30
30
Protein synthesis (30S inhibitors)
Tetracyclines
Spectinomycin
Streptomycin
Gentamicin
Kanamycin
Amikacin
Nitrofurans
Cytoplasmic membrane structure
Polymyxins
Daptomycin
PABA
Cytoplasmic membrane
Cell wall
Protein synthesis (tRNA)
Mupirocin
Puromycin