Antibiotics: mode of action and mechanisms of resistance.

Slides made by

Special consultant Henrik Hasman

Statens Serum Institut

This presentation

- Definitions needed to discuss antimicrobial resistance
- Classes of antimicrobial drugs
- Targets for antimicrobials
- Mechanisms of resistance
- Exercises

What is the difference between antibiotics and antimicrobial agents?

Antibiotics:

Naturally occurring microbial products

Antimicrobial agents:

- Any agent used to treat systemic infections
- Any agent used for disinfection
- Any compound used as an antiseptic agent

Can anybody name an antibiotic?

Can anybody name an antimicrobial agent?

70% ethanol is a product from microorganisms and kills bacteria.
Is ethanol an antibiotic?

NO!

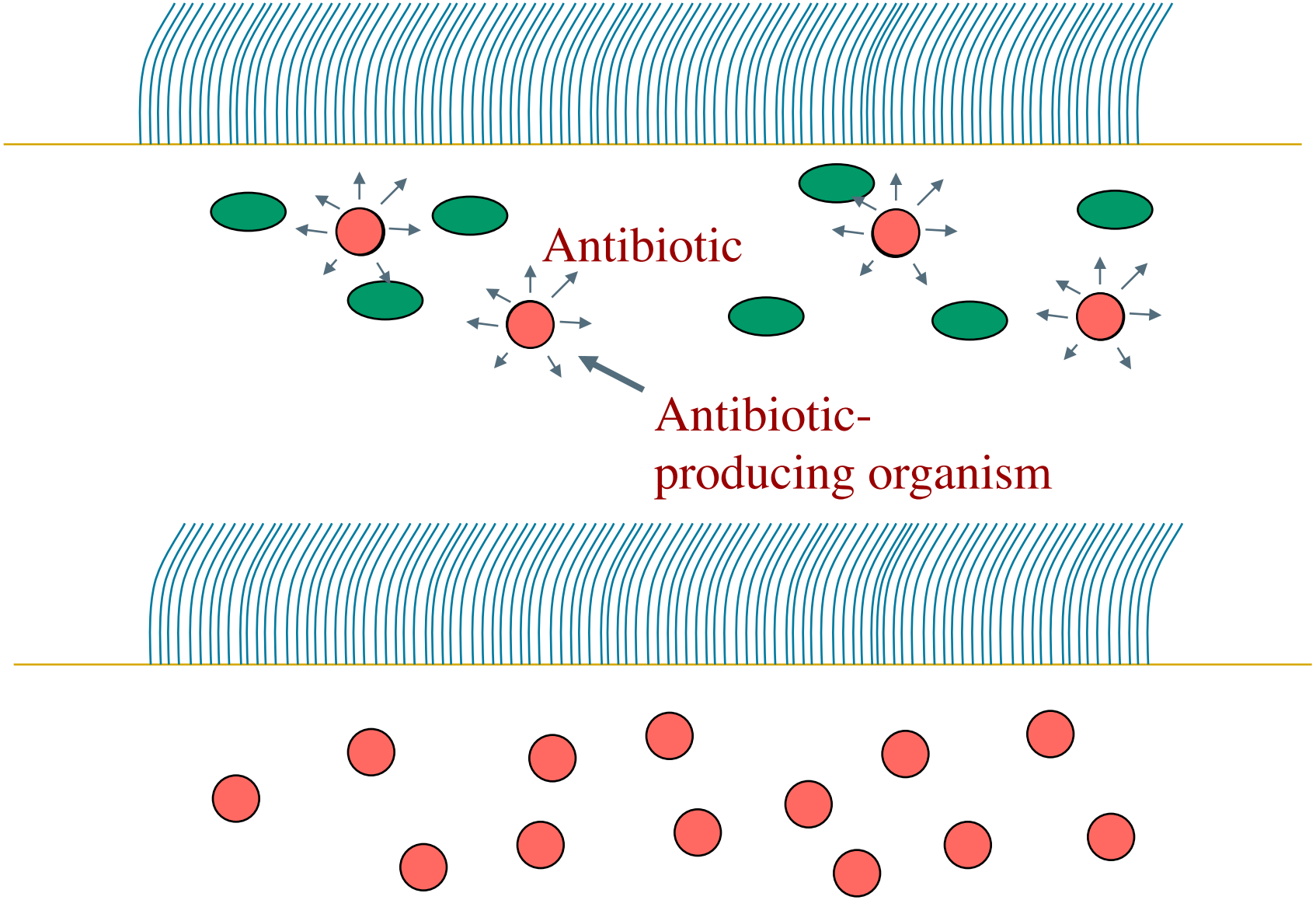
Antibiotics should not be harmful to the host in the concentrations used for treatment!

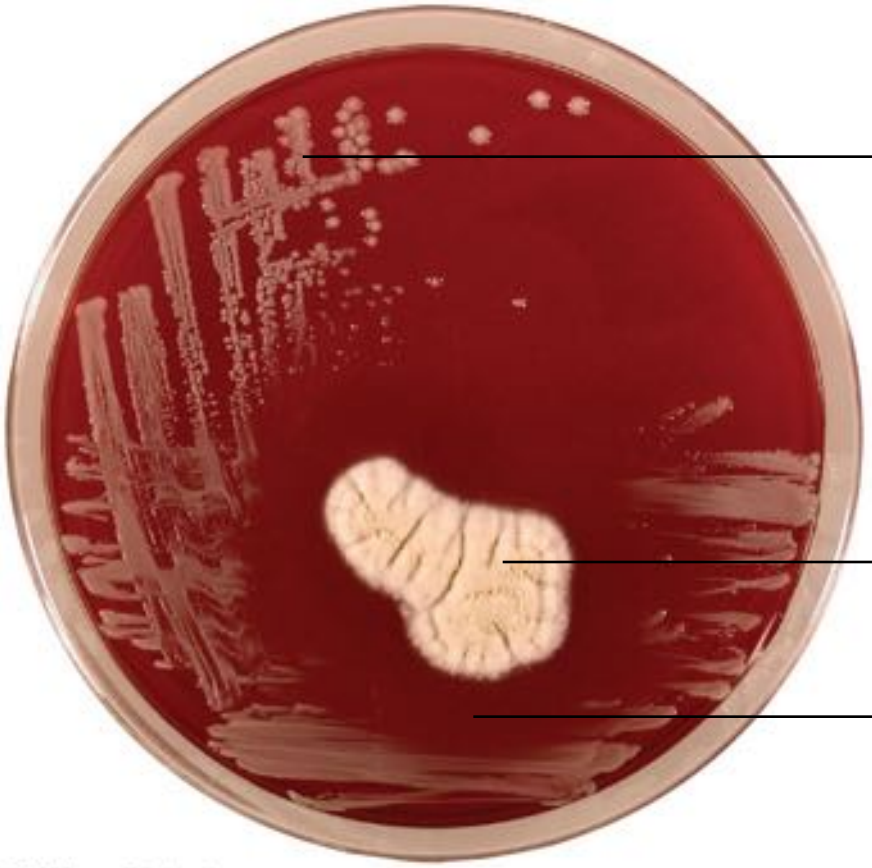
Furthermore, they should be able to enter the site of infection in therapeutic concentrations.

Why does bacteria produce antibiotics?

”Chemical warfare”

This gives the antibiotic-producing organism a growth advantage in its niche”


Antibiotic
Antibiotic-
producing organism



Staphylococcus aureus **(bacterium)**

Penicillium chrysogenum **(fungus)**

Zone where bacterial growth is inhibited

Antibiotics: The short version

Class	Origin/organism
Aminoglycosides	*Streptomyces*, *Micromonospora sp*
Cephalosporins	*Cephalosporium* sp
Macrolides	Various Actinomycetes
Penicillins	*Penicillium* sp
Phenicols	*Streptomyces venezuelae**
Quinolones	Synthetic
Rifamycins	*Amycolatopsis mediterranei*
Sulfonamides	Synthetic
Tetracyclines	*Streptomyces* sp

Mechanisms of antibiotics I

- Bacteriostatic

Stops growth of the infectious agent but does not kill it

The immune system has to kill the bug

- Bactericidal

Actively kills the infectious agent (some only growing bacteria)

Bacteriostatic antibiotic classes

- **Tetracyclines**
- **Aminoglycosides** (Gentamicin, Apramycin, Neomycin, Spectinomycin, Streptomycin)
- **Sulphonamides** (Sulphamethoxazole)
- **Macrolides** (Erythromycin)
- **Amphenicols** (Chlorphenicol, Florphenicol)
- **Trimethoprim**
- **Polymoxins** (Colistin)

Bactericidal antibiotics classes

Beta-lactams

- **Penicillins** (ampicillin, methicillin)
- **Cephalosporins** (Cefotaxime, Ceftazidime, Ceftiofur)
- **Monobactams** (Aztreonam)
- **Carbapenems** (Imipenem, Meropenem, Erthapenem)
- **Quinolones** (Nalidixan)
- **Fluoroquinolones** (Ciprofloxacin)
- **Glycopeptides** (Vancomycin)

Spectrum?

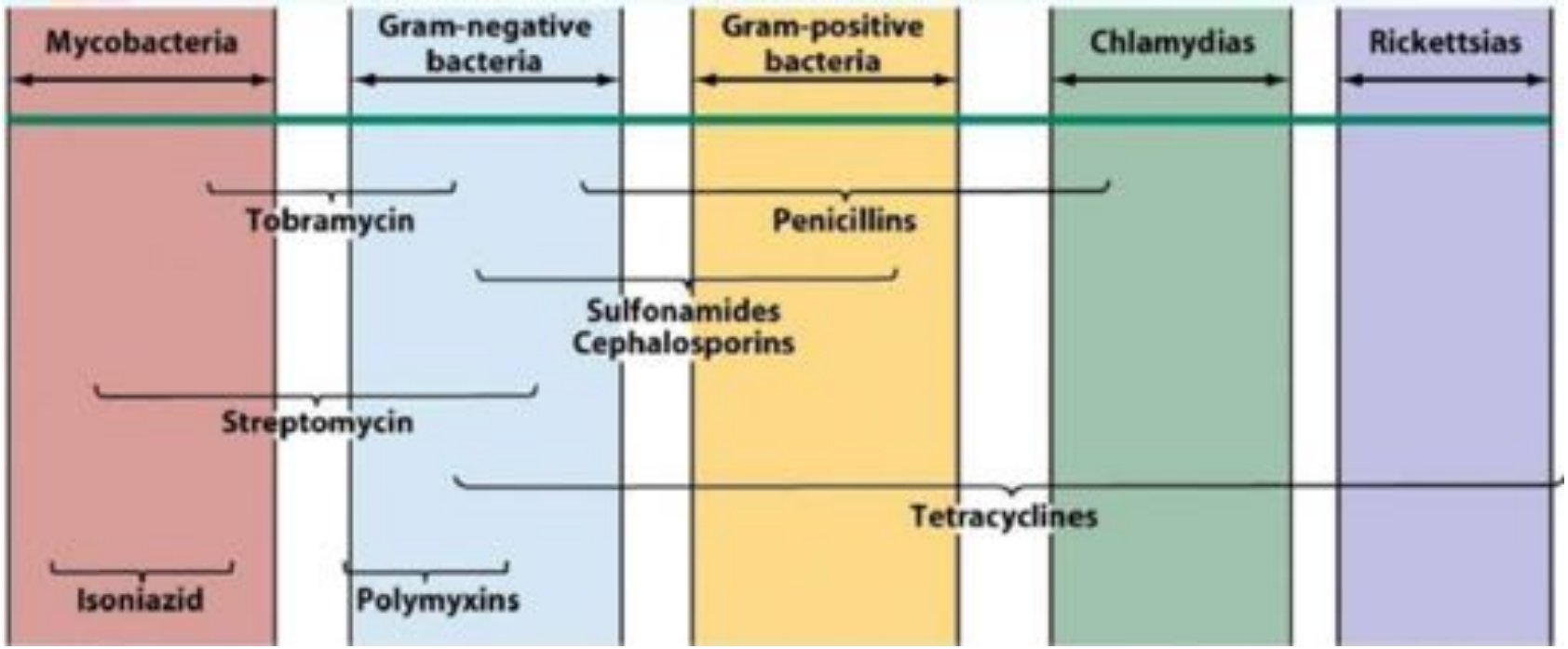
- Small spectrum

Only kills a small sub-set of bacterial species (e.g. Strep's)

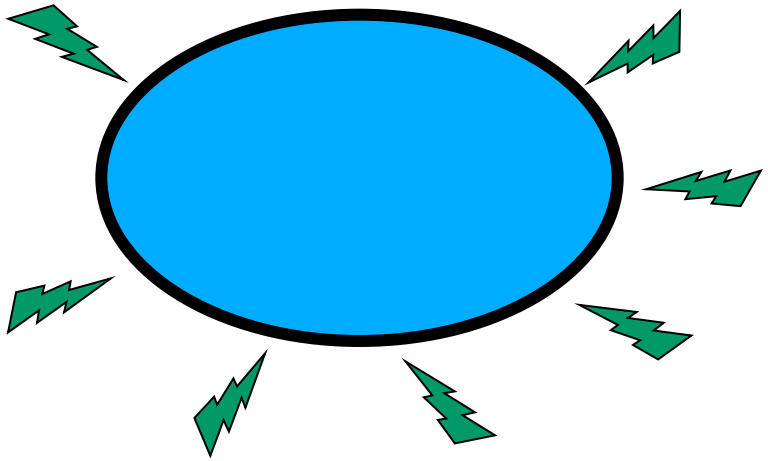
- Broad spectrum

Kills many different bacterial species including G-ve's

Spectrum of antibiotics



Which processes does antimicrobial agents interfere with in bacteria?

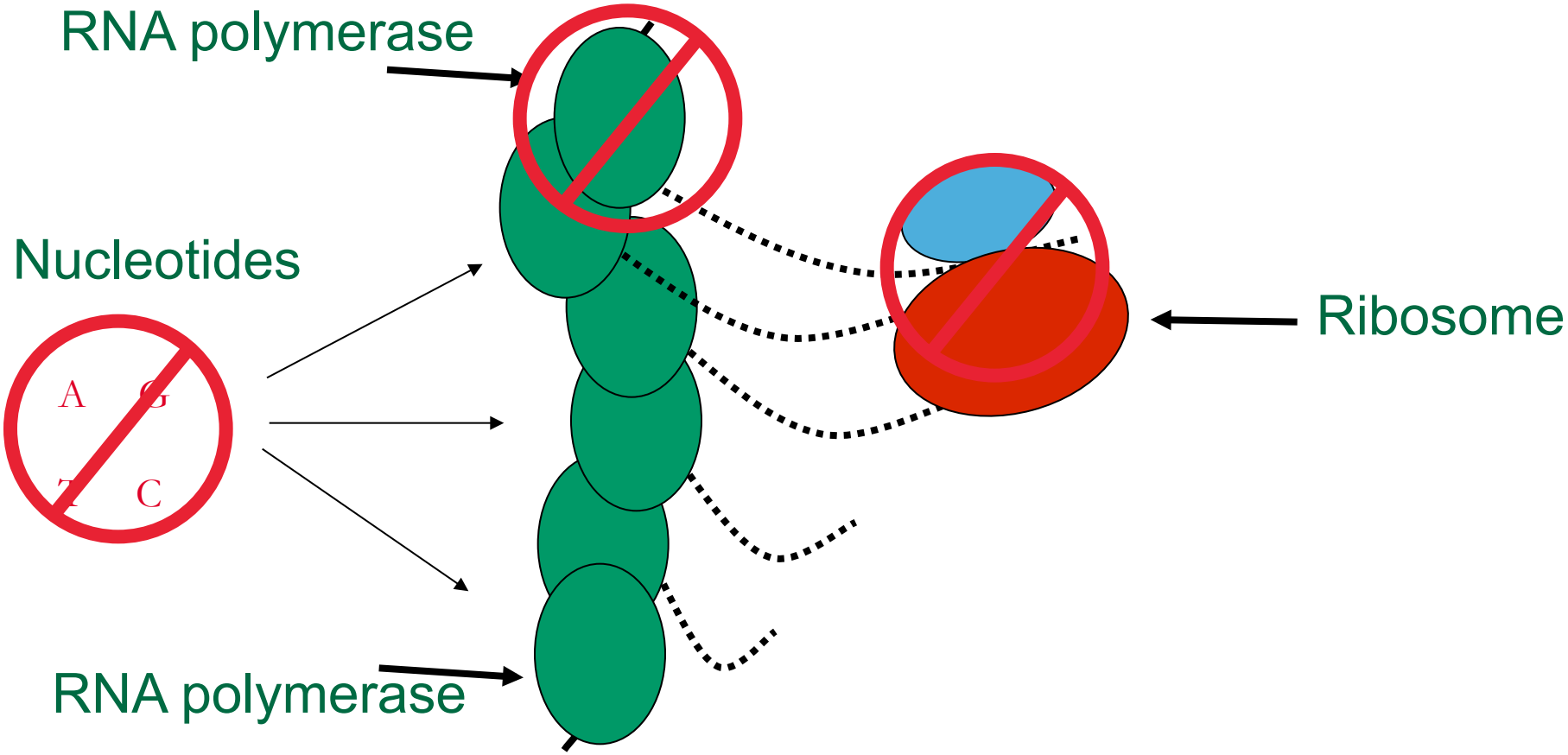


To kill a bacteria, an antimicrobial agent should hit **vital processes** in bacteria. **Can anybody name at least one** such process?

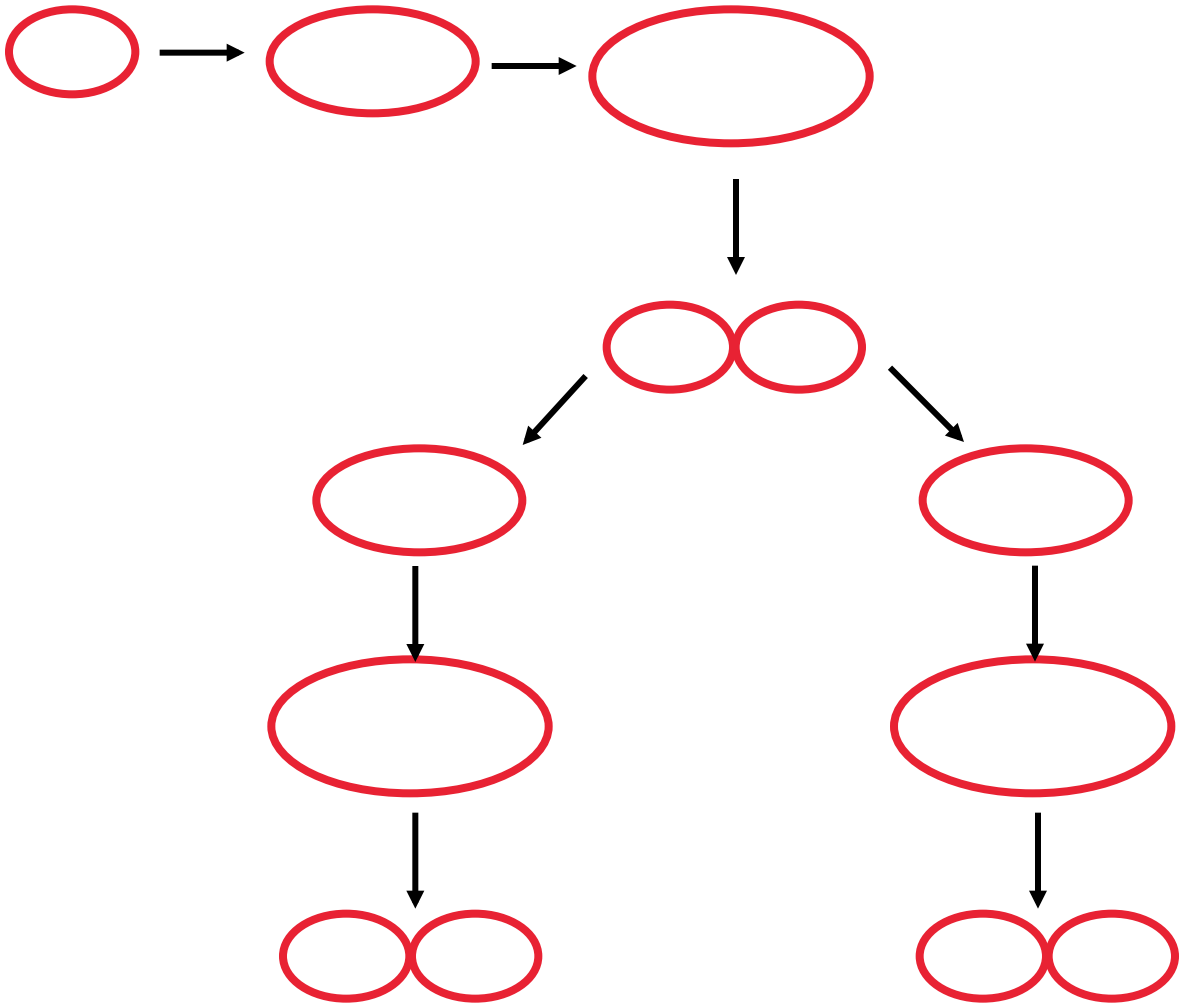
Antibiotics: Modes of action

- Inhibitors of DNA synthesis
- Inhibitors of bacterial protein synthesis
- Inhibitors of bacterial cell wall synthesis

From DNA to protein

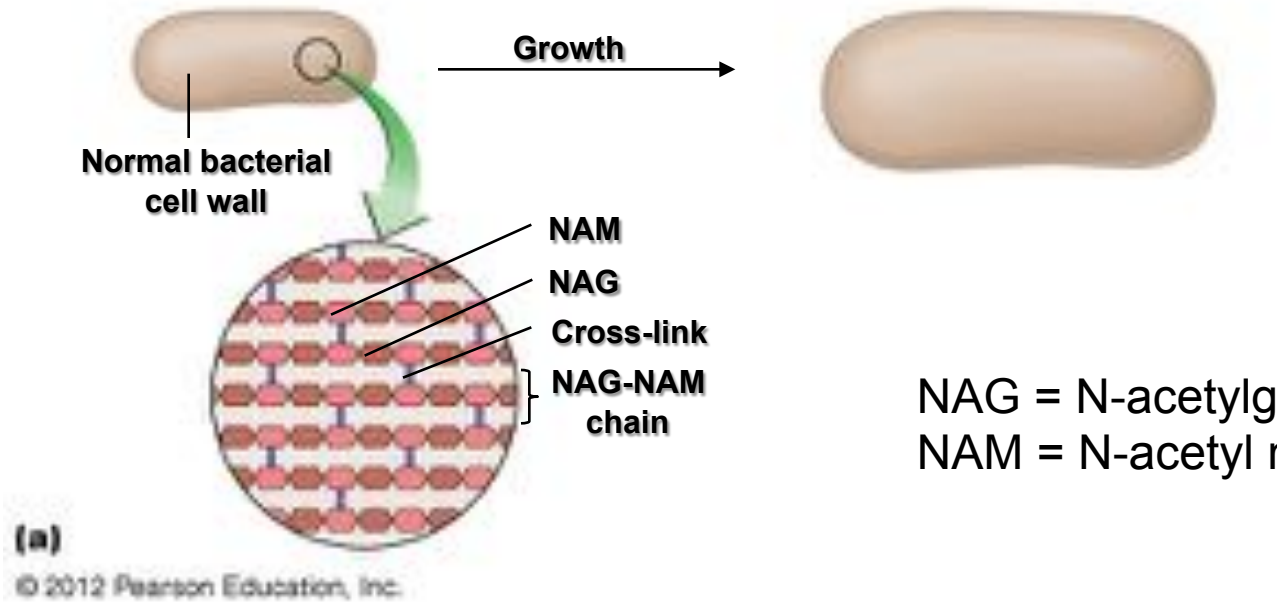


Bacterial growth



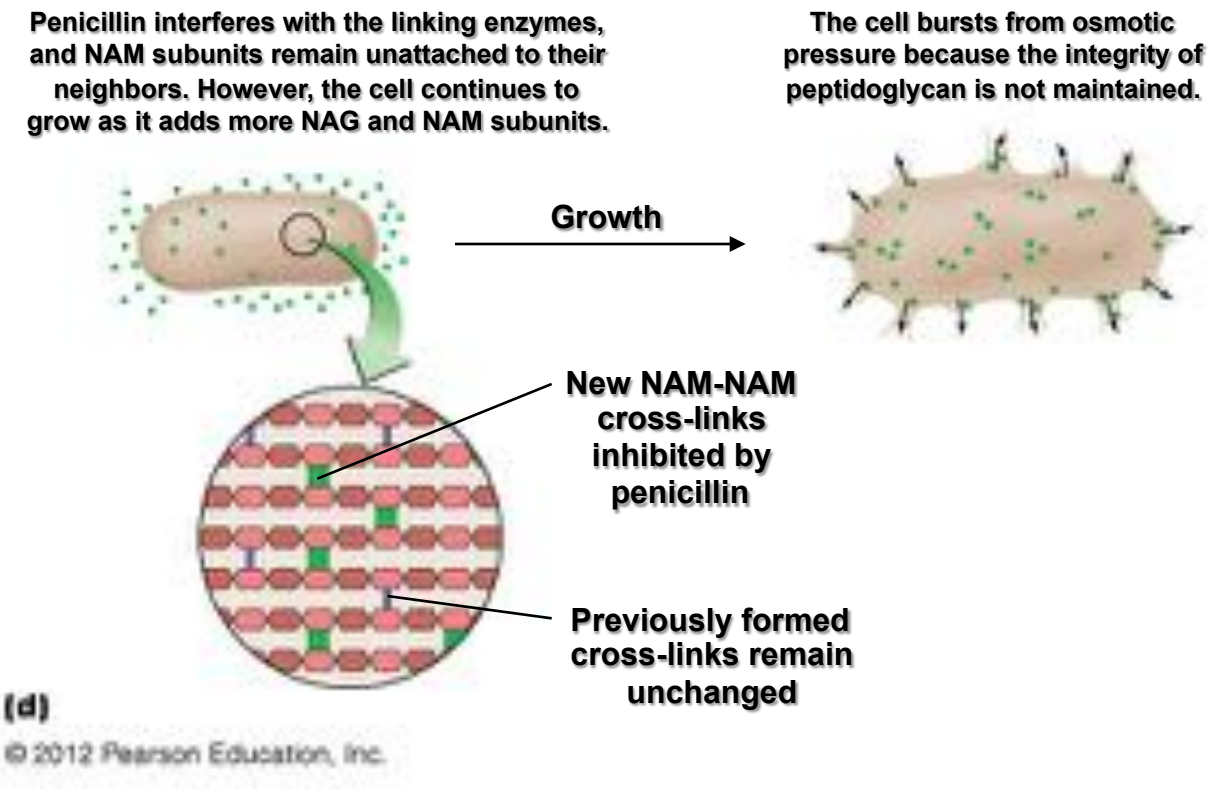
A bacterial cell wall is composed of a macro-molecule of peptidoglycan composed of NAG-NAM chains that are cross-linked by peptide bridges between the NAM subunits.

New NAG and NAM subunits are inserted into the wall by enzymes, allowing the cell to grow. Normally, other enzymes link new NAM subunits to old NAM subunits with peptide cross-links.

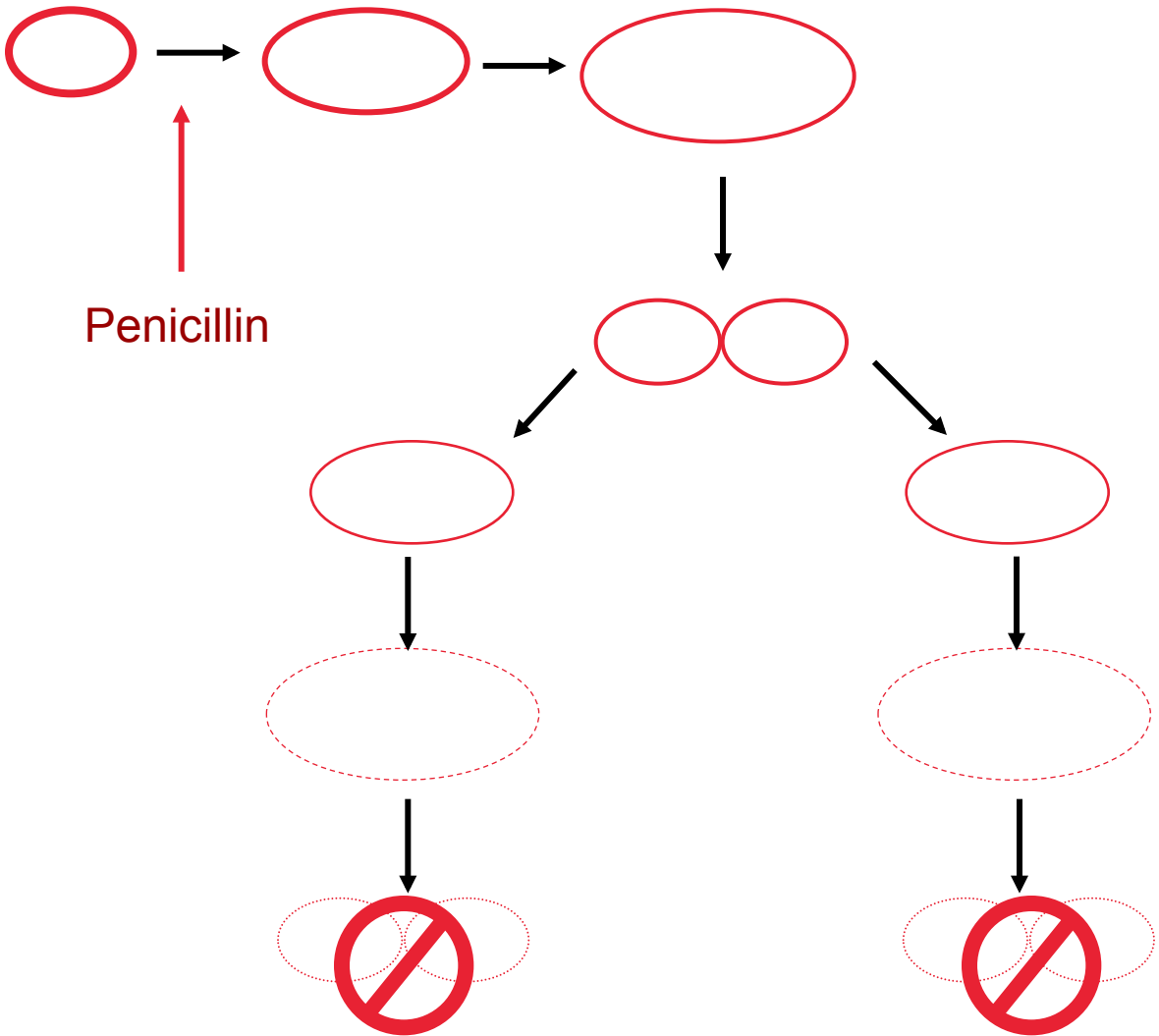


NAG = N-acetylglucosamine
NAM = N-acetyl muramic acid

Figure 10.3d The effect of penicillin on peptidoglycan in preventing NAM-NAM cross-links



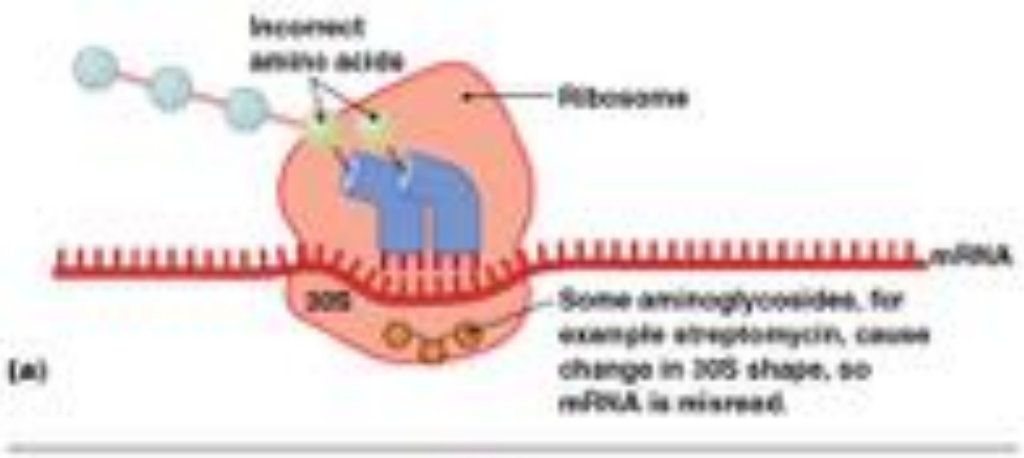
Inhibition of cell wall synthesis



- **Inhibition of Protein Synthesis**
  - Prokaryotic ribosomes are 70S (30S and 50S)
  - Eukaryotic ribosomes are 80S (40S and 60S)
  - Drugs can selectively target translation
  - Mitochondria of animals and humans contain 70S ribosomes
    - Therefore, antimicrobials interacting with the 70S can be harmful if they are able to penetrate the host (eukariotic) cells

Antimicrobials that inhibit protein synthesis

- 30 S subunit
  - Aminoglycosides such as streptomycin and gentamicin
  - Tetracyclines
- 50 S subunit
  - Chloramphenicol, lincosamides, streptogramins, and macrolides such as erythromycin


Incorrect amino acids
Ribosome
mRNA
30S
Some aminoglycosides, for example streptomycin, cause change in 30S shape, so mRNA is misread.
(a)

- **Inhibition of Nucleic Acid Synthesis**
  - Quinolones (Naldixic acid) and fluoroquinolones (Ciprofloxacin)
    - Act against prokaryotic DNA gyrase (part of the DNA replication machinery)

DNA gyrase – (Fluoro-) quinolones

Antibiotics

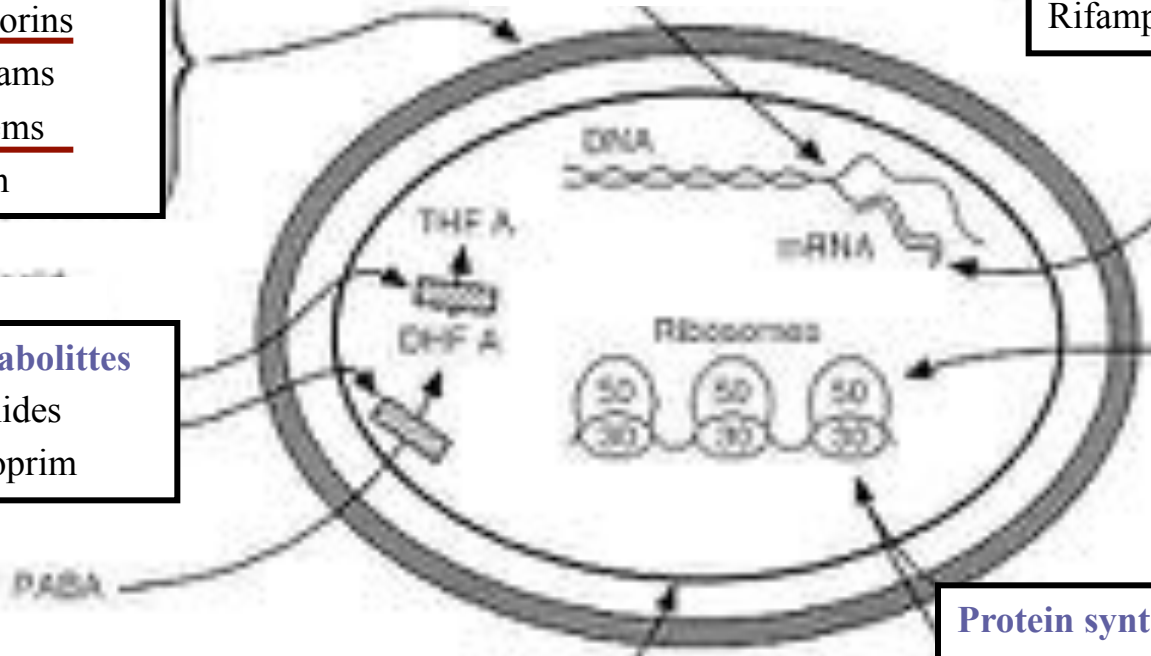
Cell wall synthesis
β-lactams:
Penicillin
Cephalosporins
Monobaktams
Carbapenems
Vancomycin

DNA replication
Fluoquinolones
Metronidazole

RNA-polymerase
Rifampicin

Protein syntese Inhibitors (50s)
Macrolides
chloramphenikol
Clindamycin

Anti metabolittes
Sulfonamides
Trimethroprim



Cell membrane
Polymyxin
Amphotericin

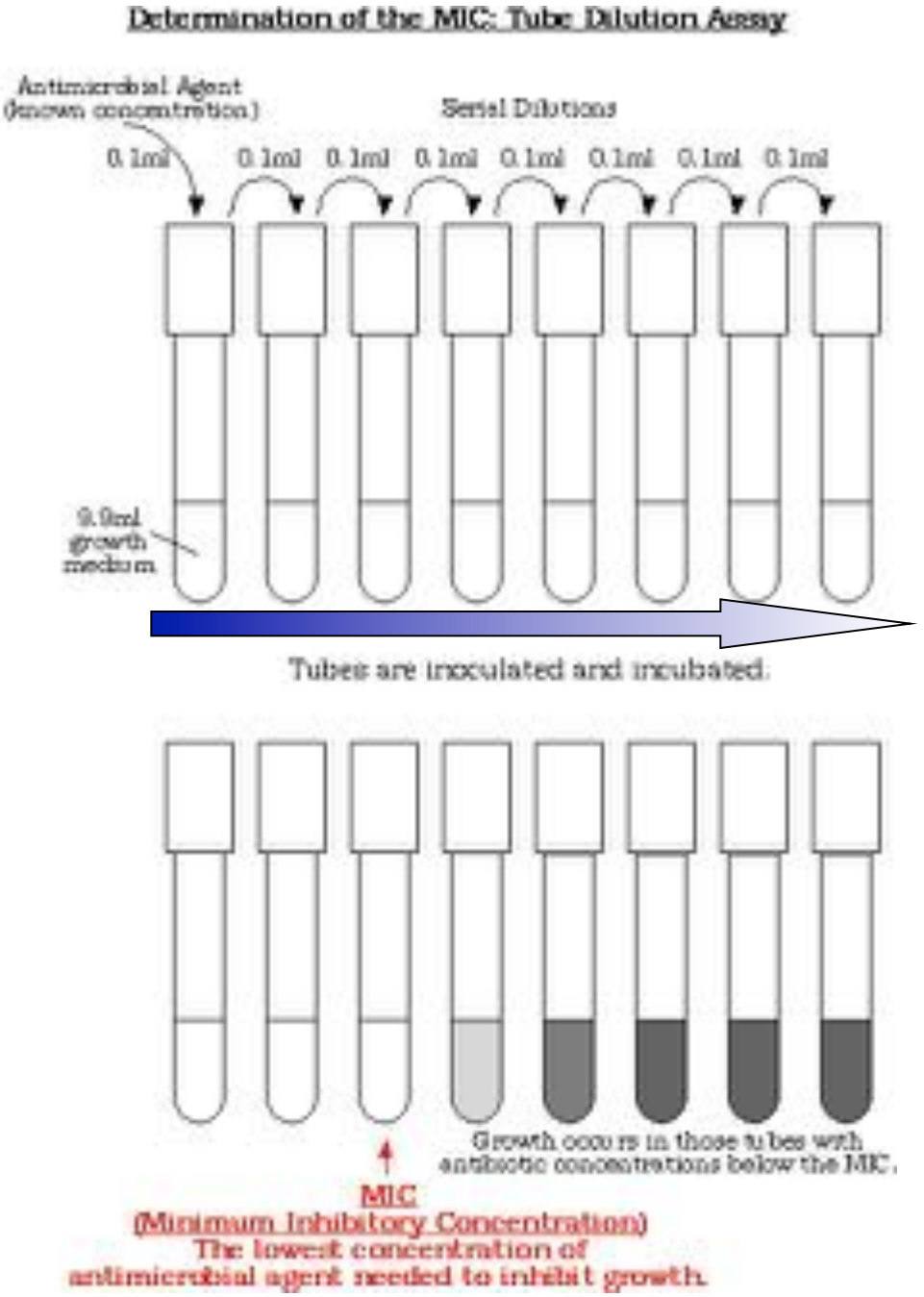
Protein syntese Inhibitors (30s)
Tetracyclines
Aminoglykocides
Fucidinic acid

BREAK

How do we measure the effect of an antimicrobial agent against a given pathogen?

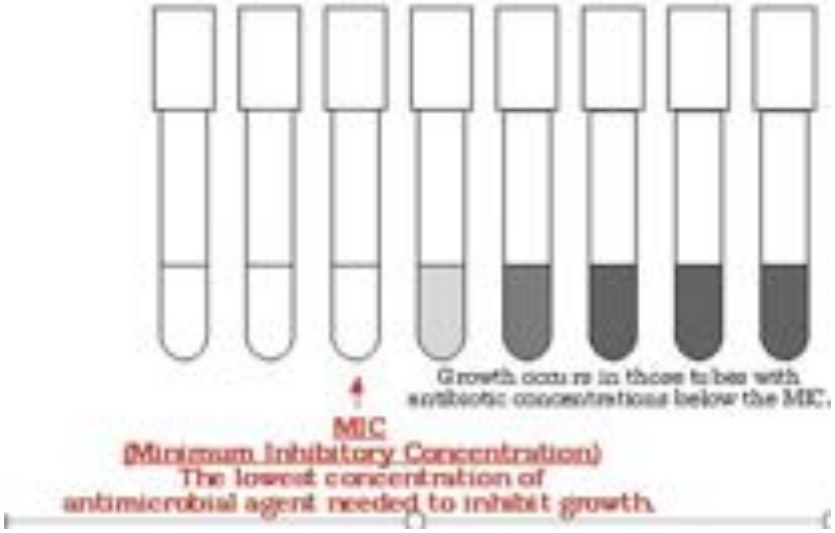
Dilution method

MIC



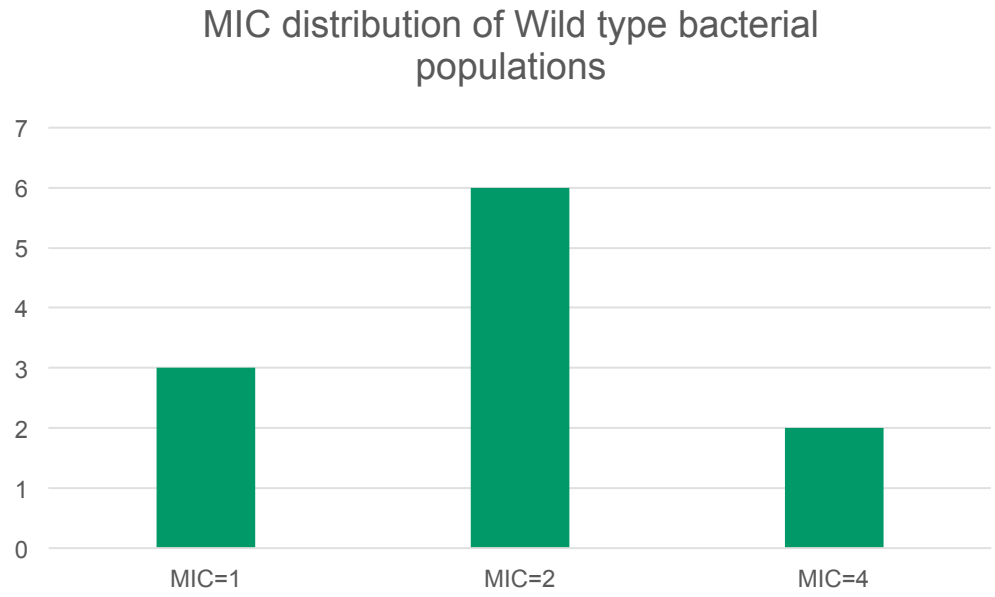
Question

- How can we discriminate between biocidal and biostatic antimicrobial agents in a MIC experiment?

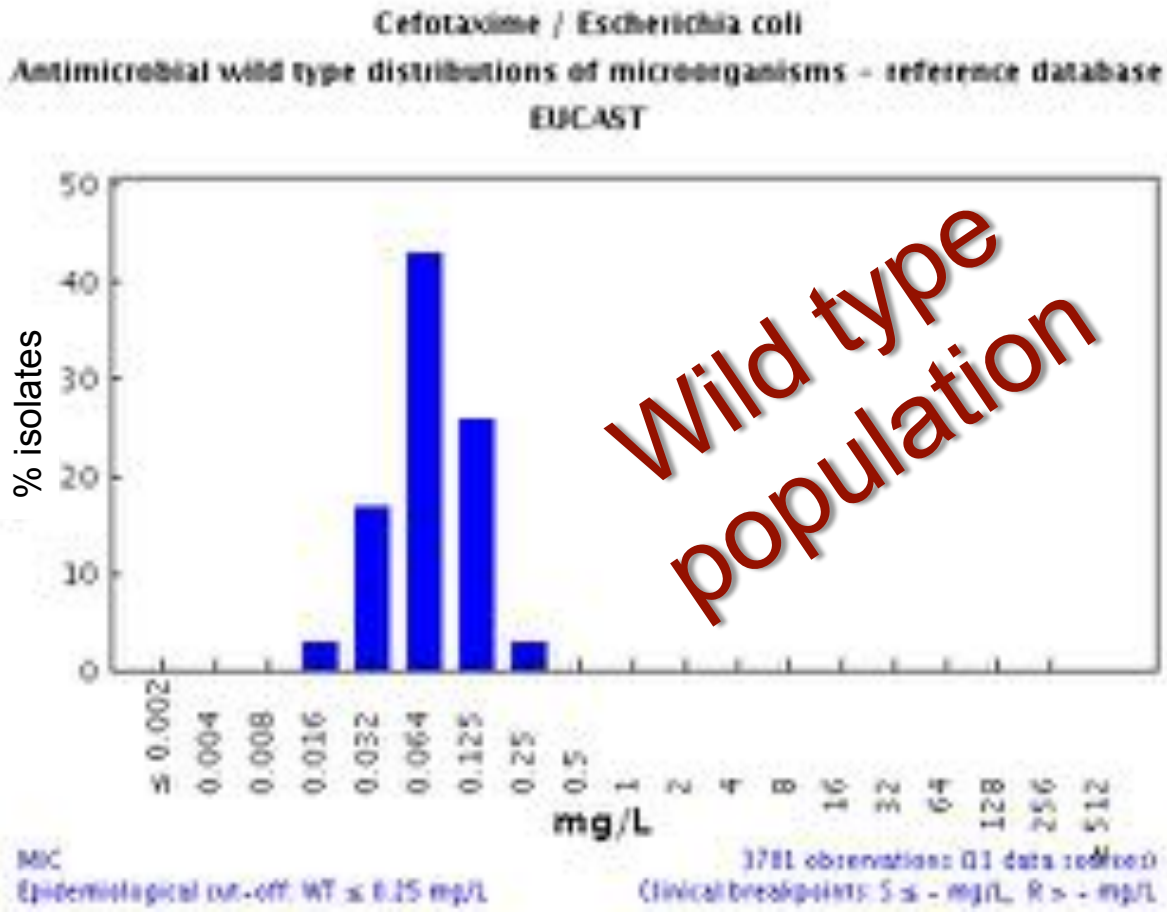


MIC results – Wild type population

Name
Isolate 1
Isolate 2
Isolate 3
Isolate 4
Isolate 5
Isolate 6
Isolate 7
Isolate 8
Isolate 9
Isolate 10
Isolate 11



Cefotaxime susceptibility testing in *E. coli*



What is antimicrobial resistance?

superbugs

Microorganisms with multiply resistance

- **MRSA** methicillin-resistant Staphylococcus aureus
- **VISA** vancomycin intermediate resistant Staphylococci
- **VRE** vancomycin-resistant enterococci
- **ESBLs** extended-spectrum beta-lactamases (microorganisms – resistant to cephalosporins)

1952 – 100 % Staphylococcus infections were cured by penicillin

1982 – only 10 % infections

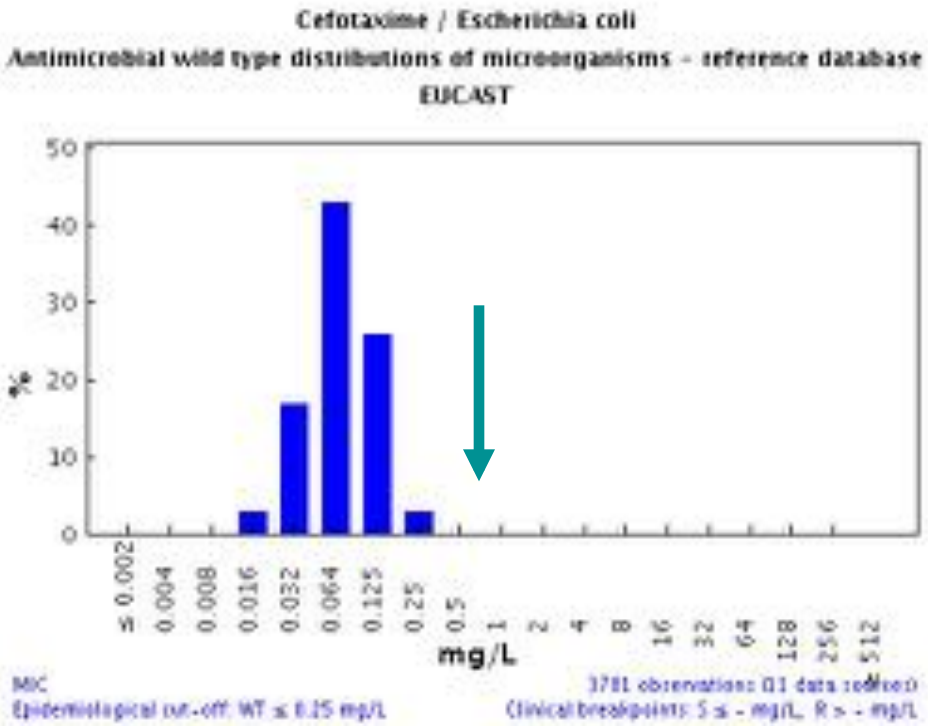
At nowadays ?.....

MRSA causes 19 000 deaths annually in USA

What is antimicrobial resistance I?

The ab *survive*
at a give *microbial*
agent at *ion of the*
m *ed*

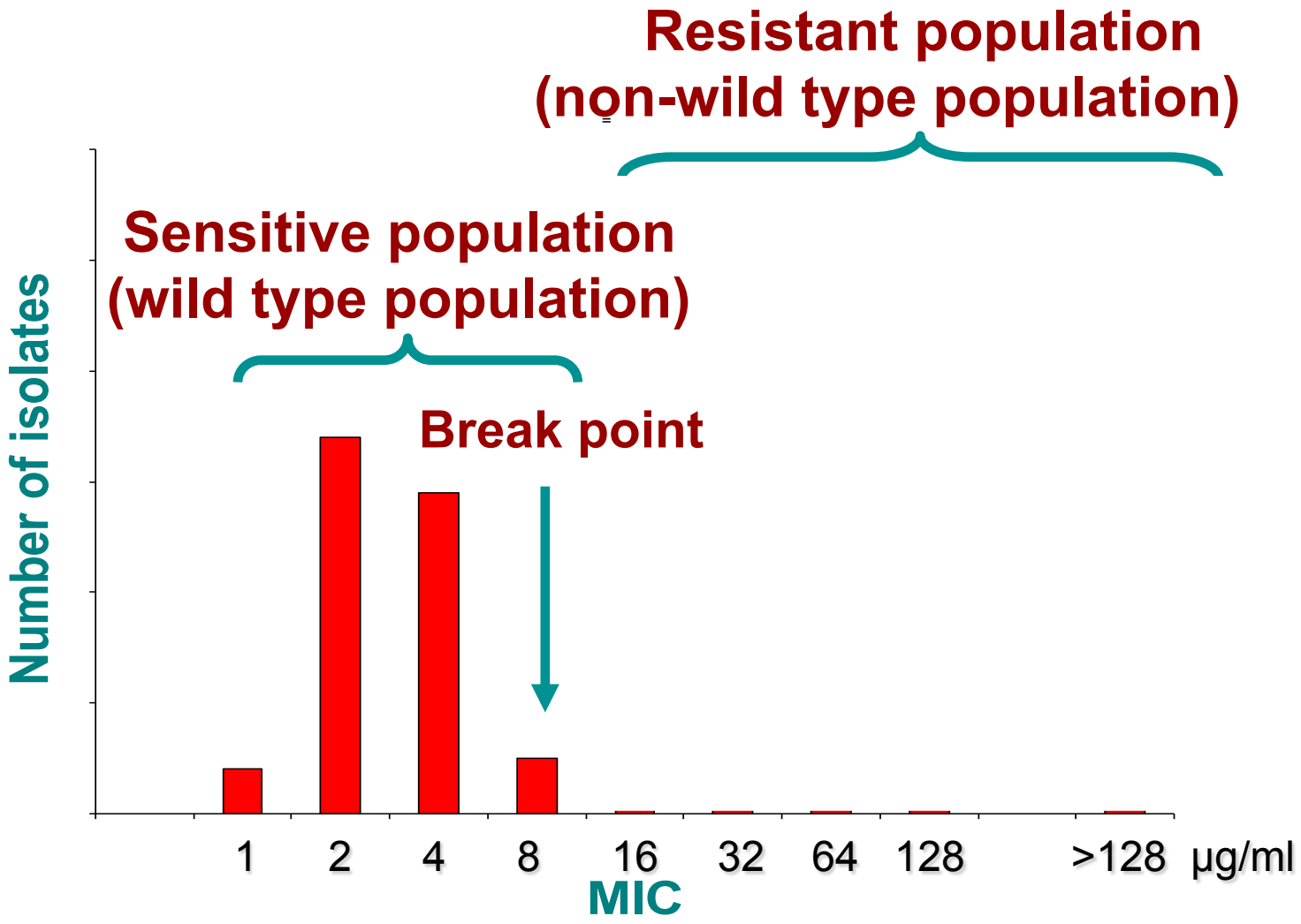
“mi **t”.**



EUCAST* is defining the microbiological breakpoints.

*European Committee on Antimicrobial Susceptibility Testing

Population distribution



MIC > Breakpoint → Resistant (R > 8 or R ≥ 16)