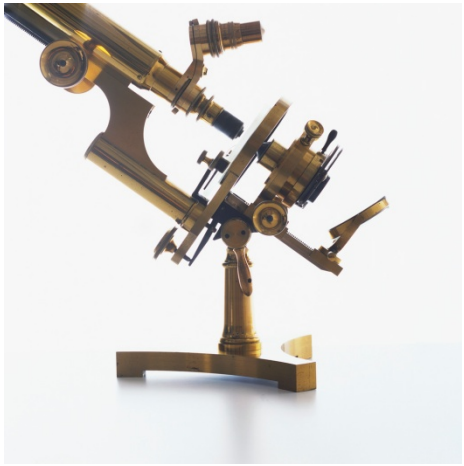
Antibiotics: mode of action and mechanisms of resistance.

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!

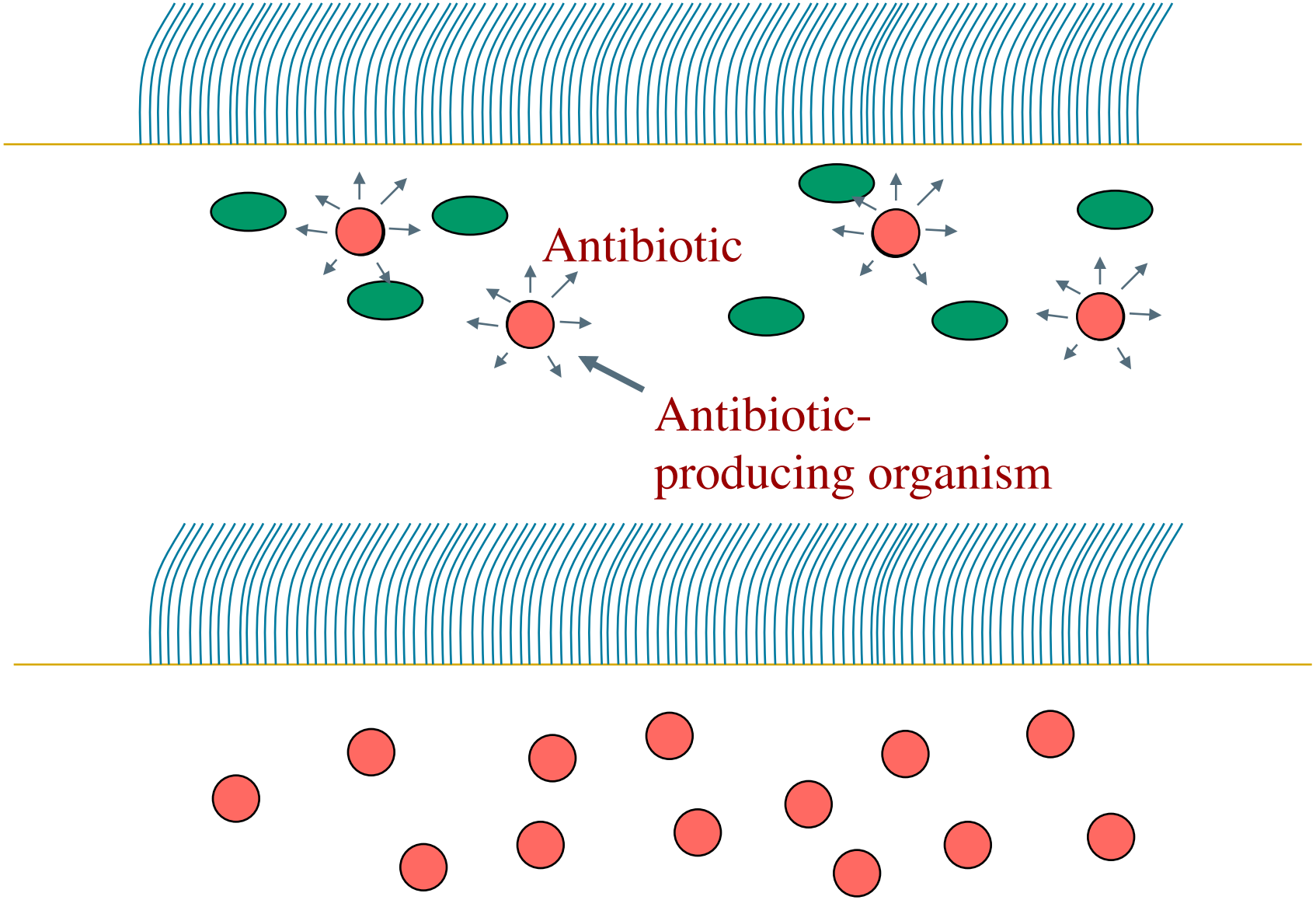
Antibiotics should not be harmful to the host in the concentrations used for treatment!

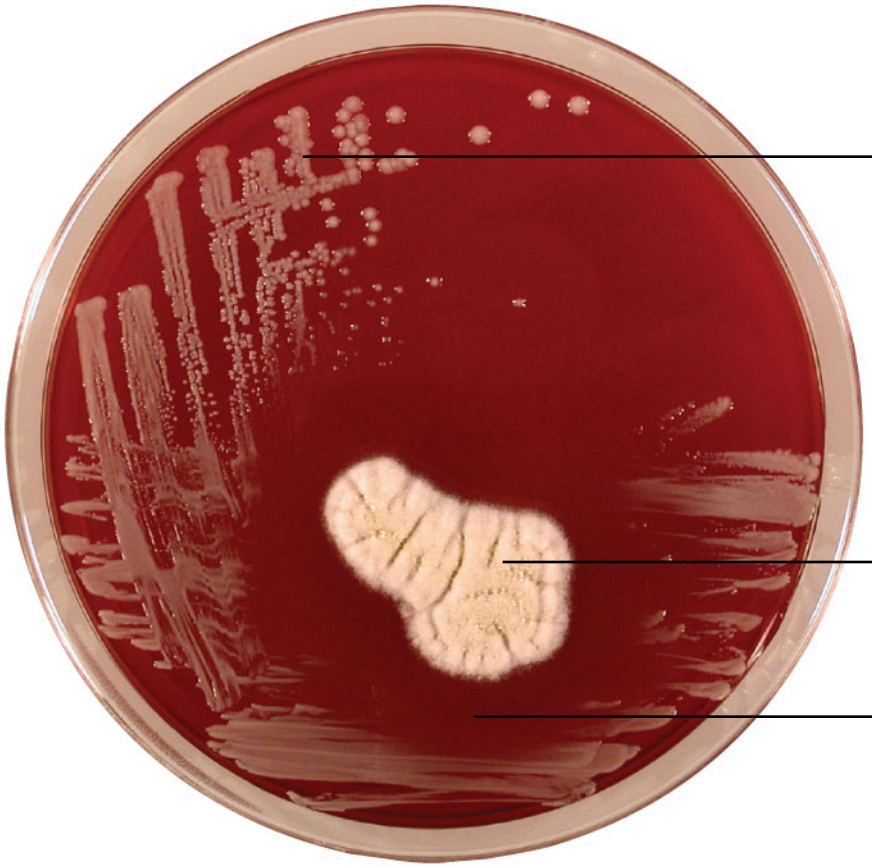
Furthermore, they should be able to enter the site of infection in therapeutic concentrations.

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?

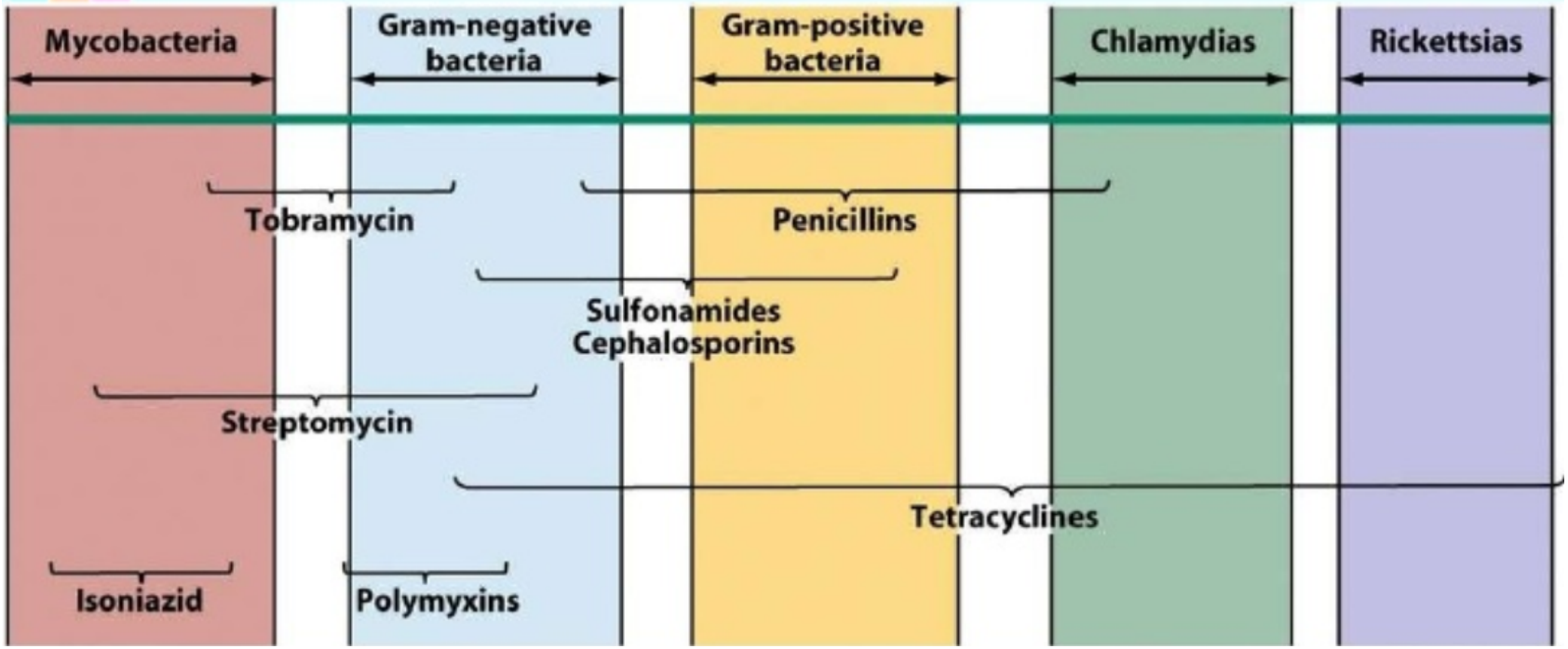
- Small spectrum

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

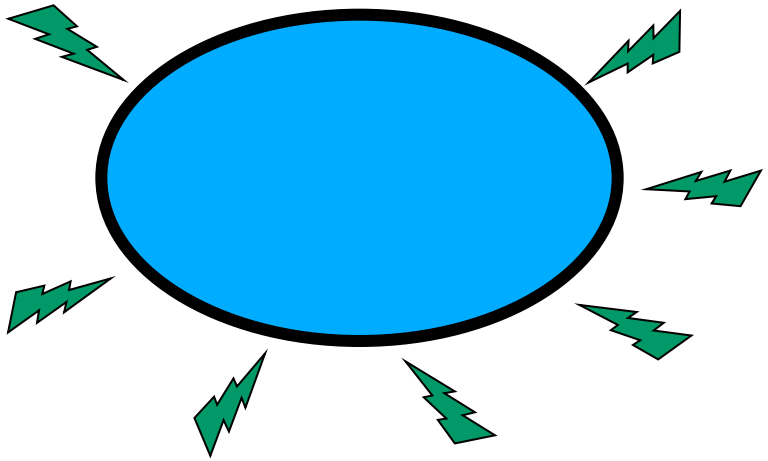
- Broad spectrum

Kills many different bacterial species including G-ve's

Spectrum of antibiotics



Which processes does antimicrobial agents interfere with in bacteria?

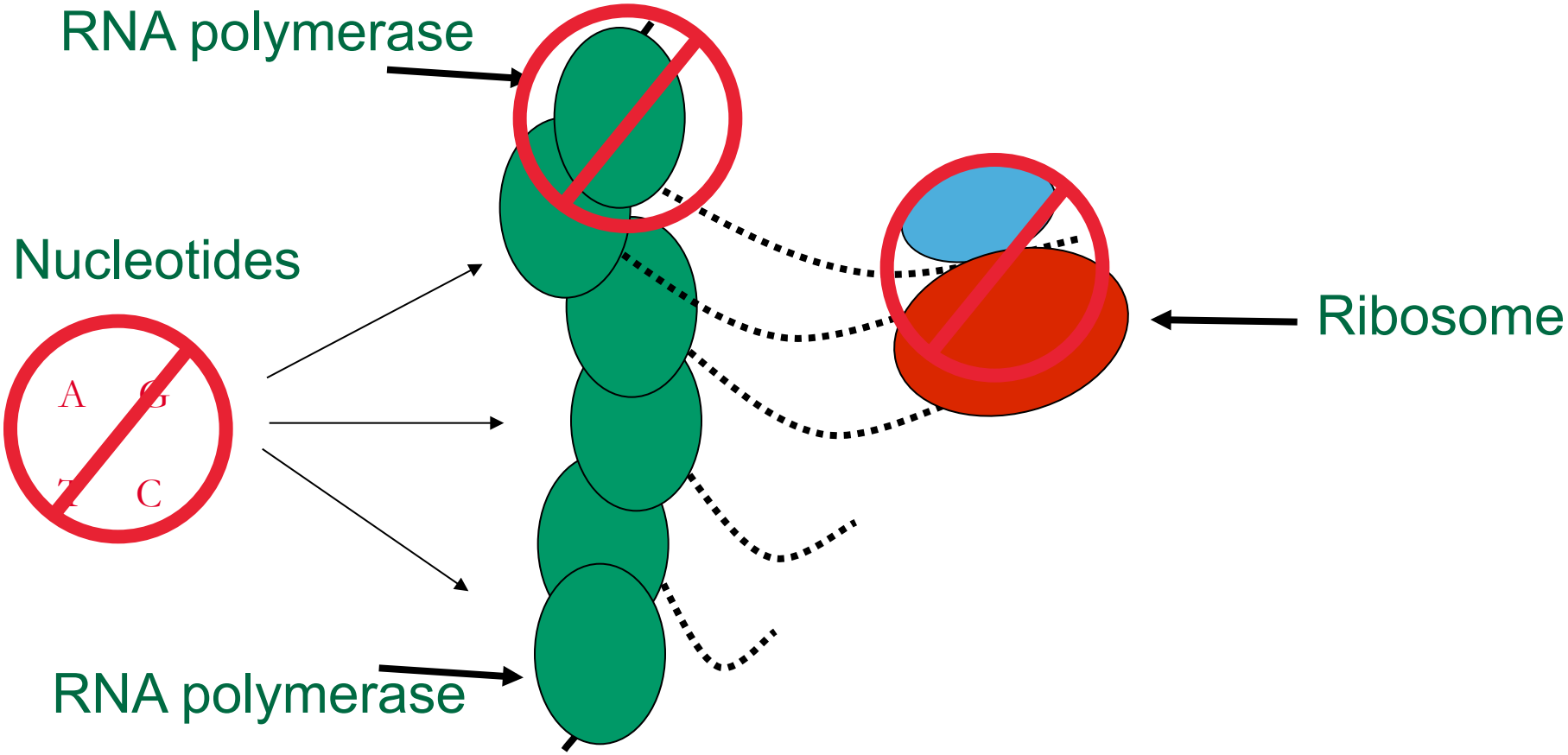


To kill a bacteria, an antimicrobial agent should hit **vital processes** in bacteria. **Can anybody name at least one** such process?

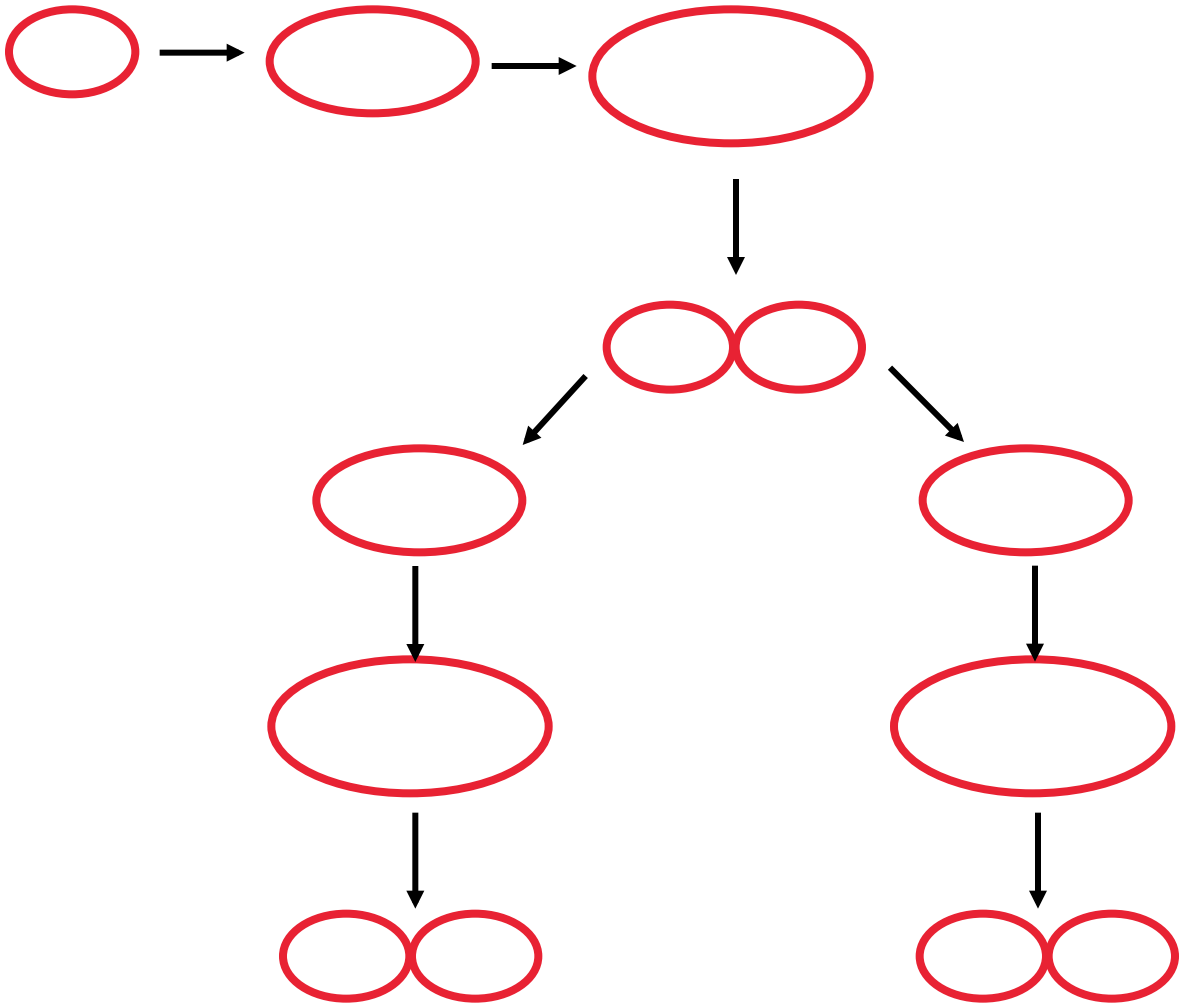
Antibiotics: Modes of action

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

From DNA to protein

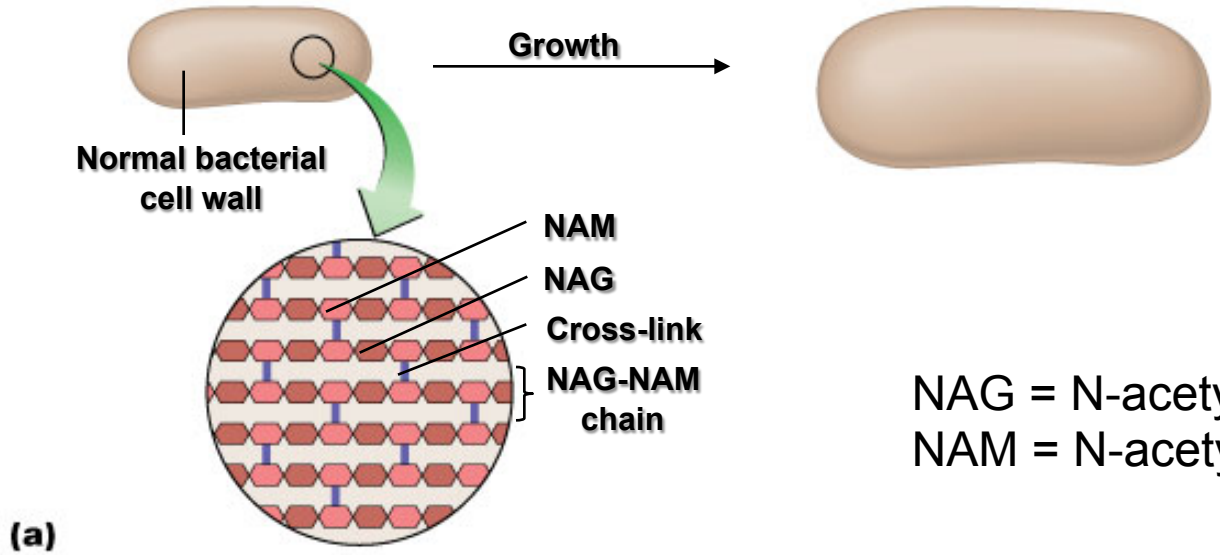


Bacterial growth



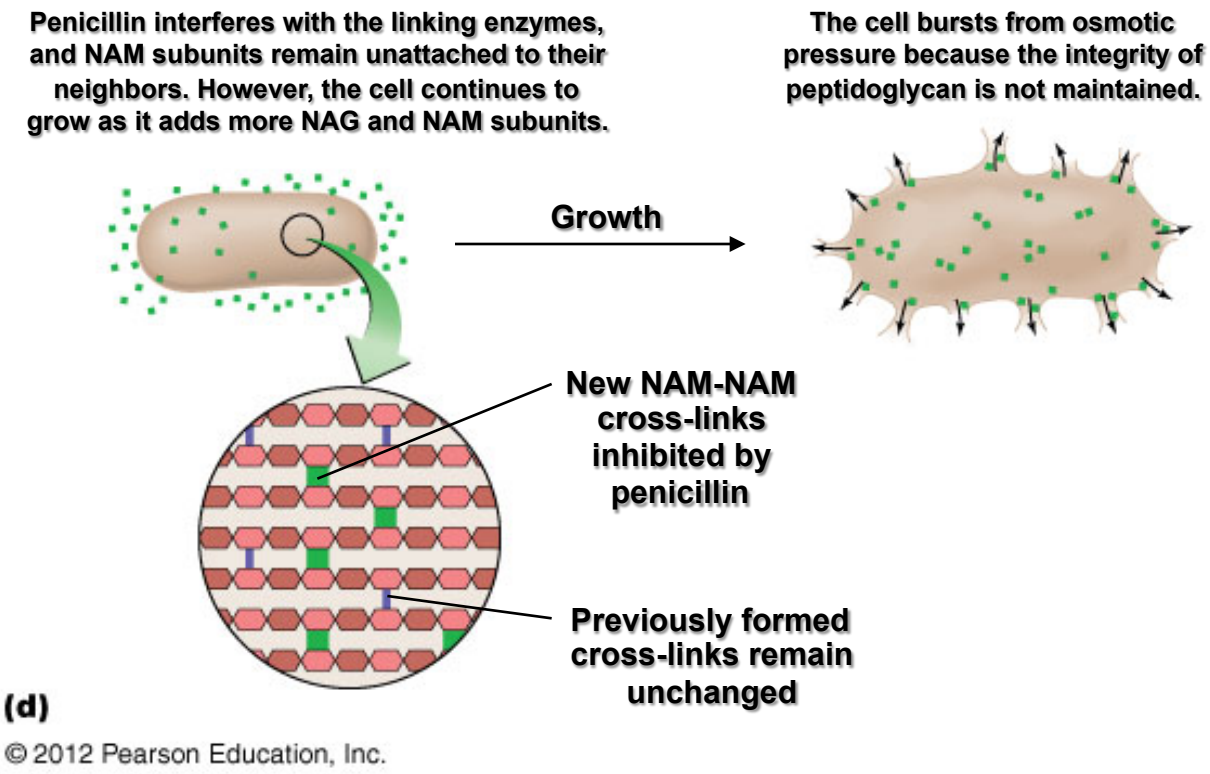
A bacterial cell wall is composed of a macro-molecule of peptidoglycan composed of NAG-NAM chains that are cross-linked by peptide bridges between the NAM subunits.

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.

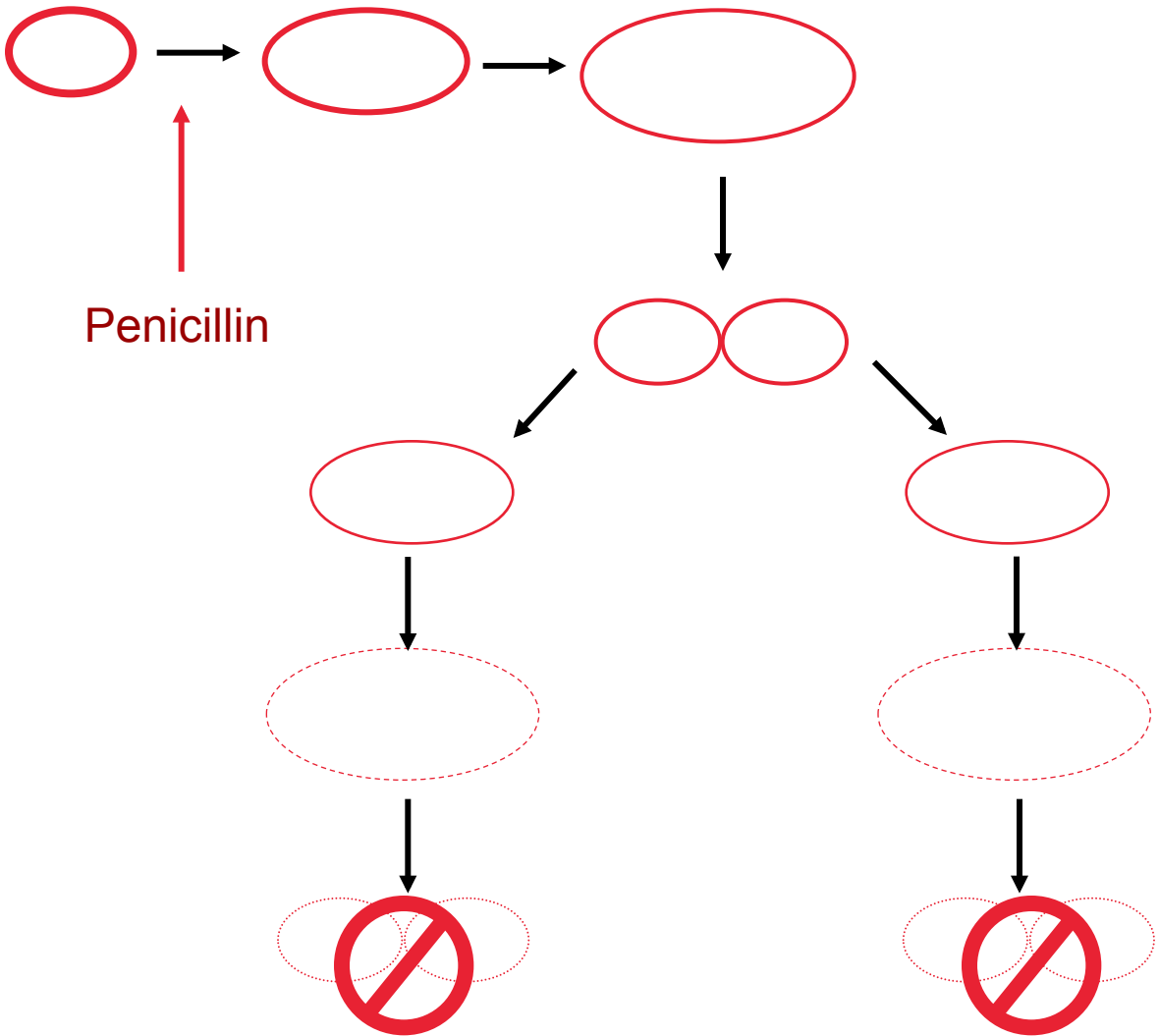


NAG = N-acetylglucosamine
NAM = N-acetyl muramic acid

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



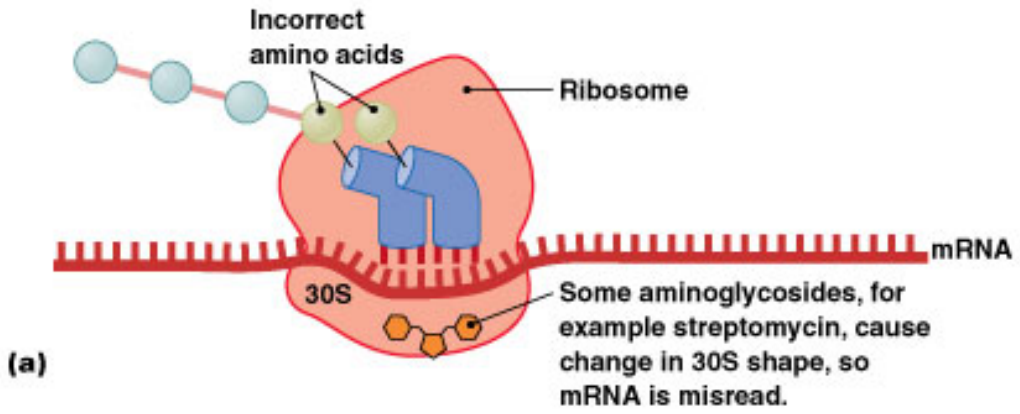
Inhibition of cell wall synthesis



- **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)

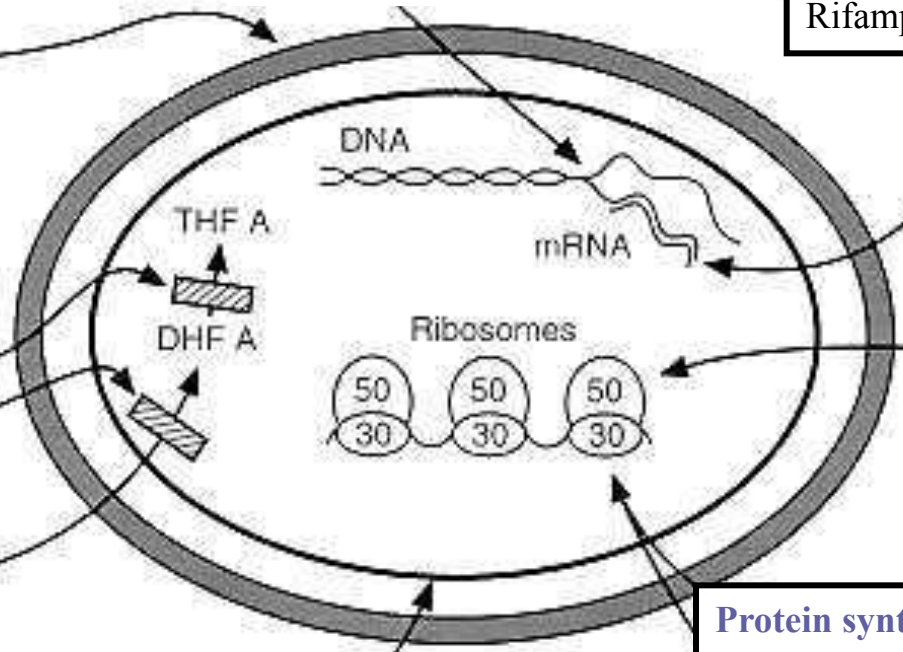
DNA gyrase – (Fluoro-) quinolones

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

Protein syntese Inhibitors (30s)
Tetracyclines
Aminoglykocides
Fucidinic acid

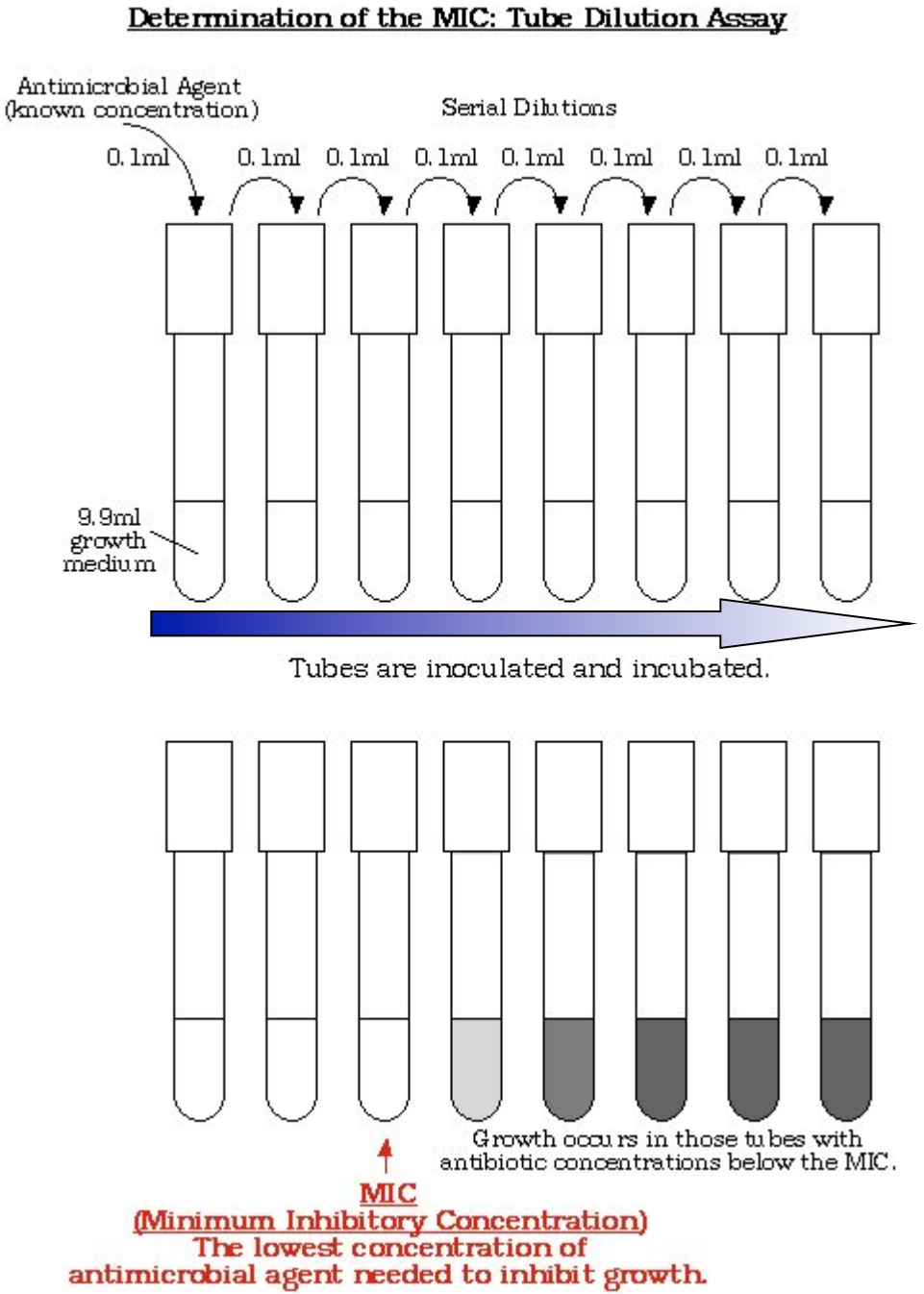
Cell membrane
Polymyxin
Amphotericin

BREAK

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

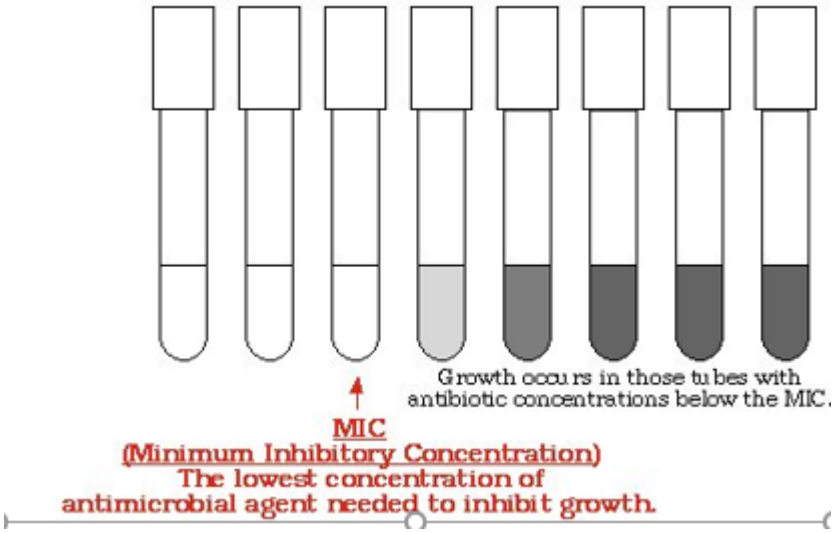
Dilution method

MIC



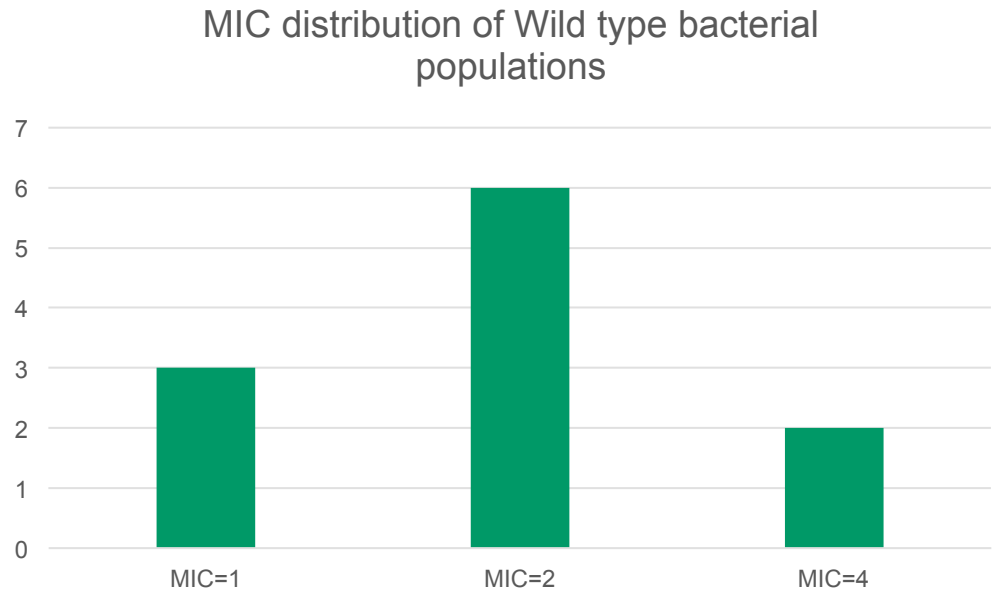
Question

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

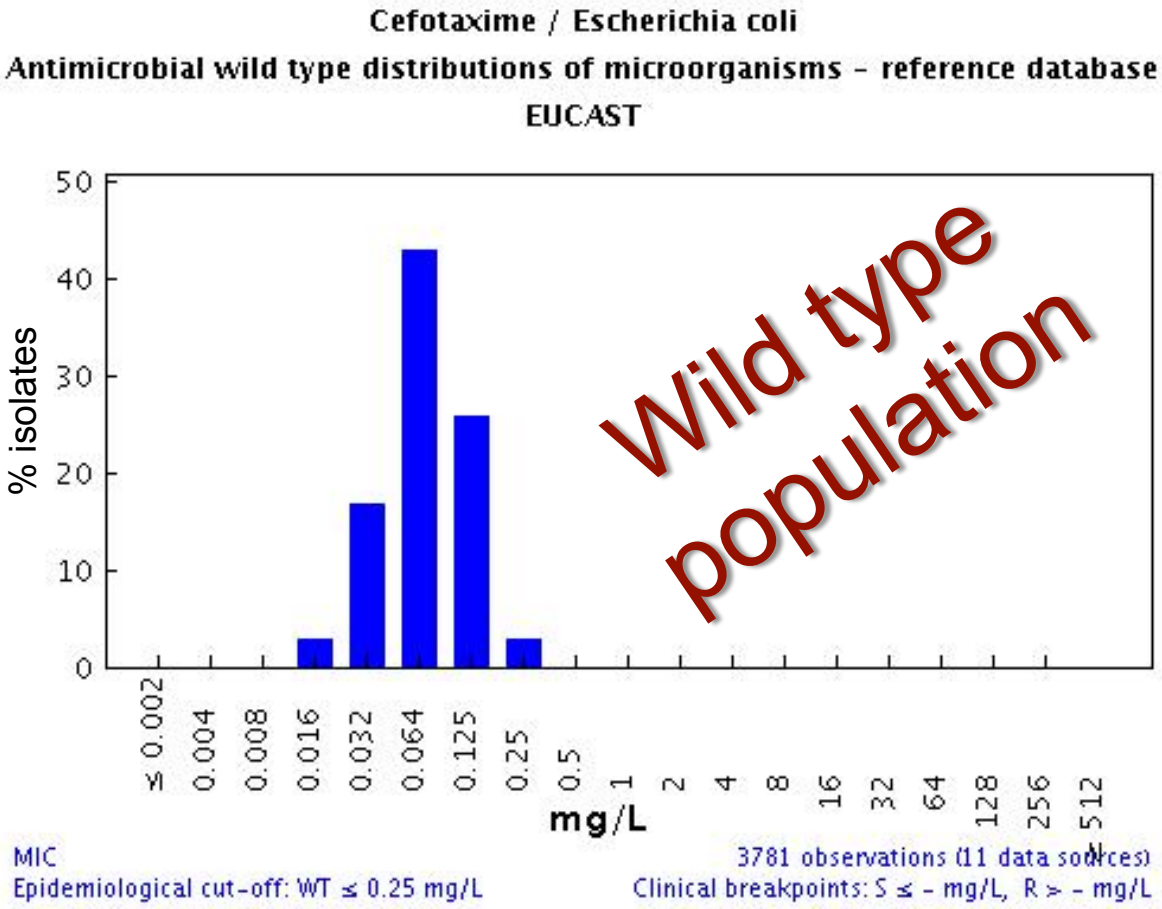


MIC results – Wild type population

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

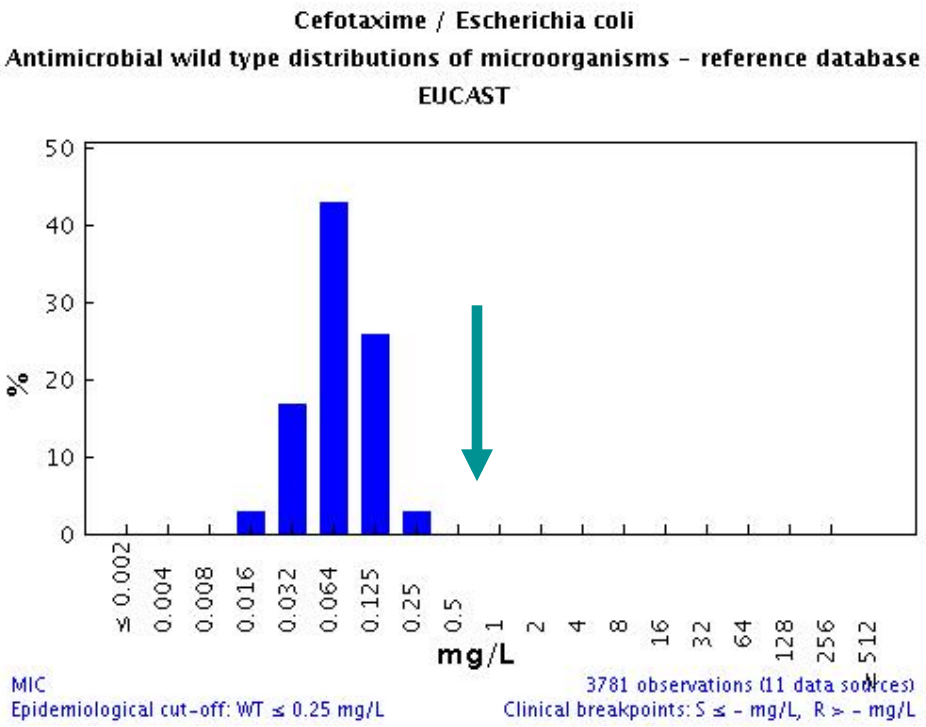
At nowadays ?.....

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*

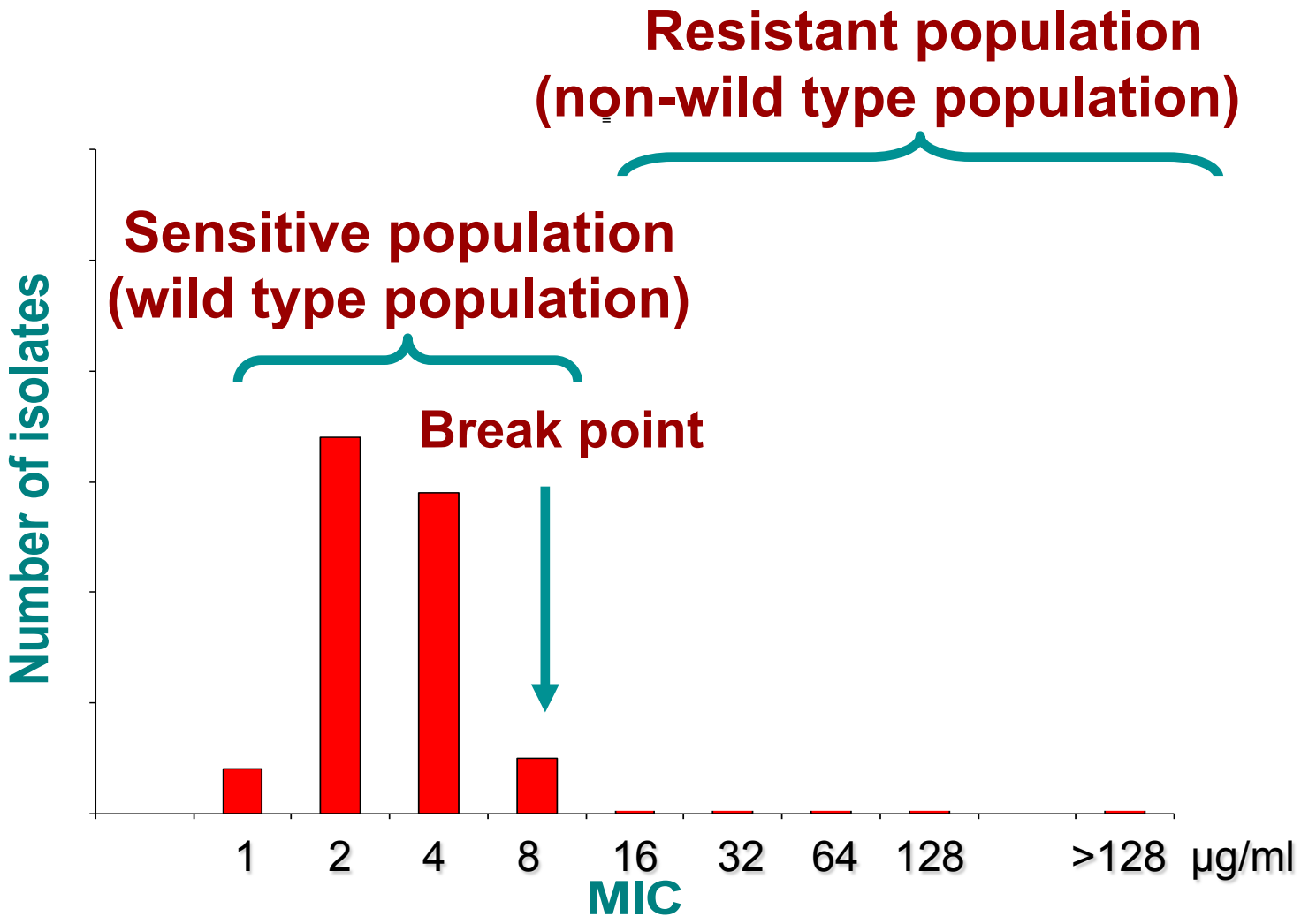
“mi **t”.**



EUCAST* is defining the microbiological breakpoints.

*European Committee on Antimicrobial Susceptibility Testing

Population distribution



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

What is antimicrobial resistance II?

The ability of a microorganism to survive treatment with a clinical concentration of an antimicrobial agent in the body.

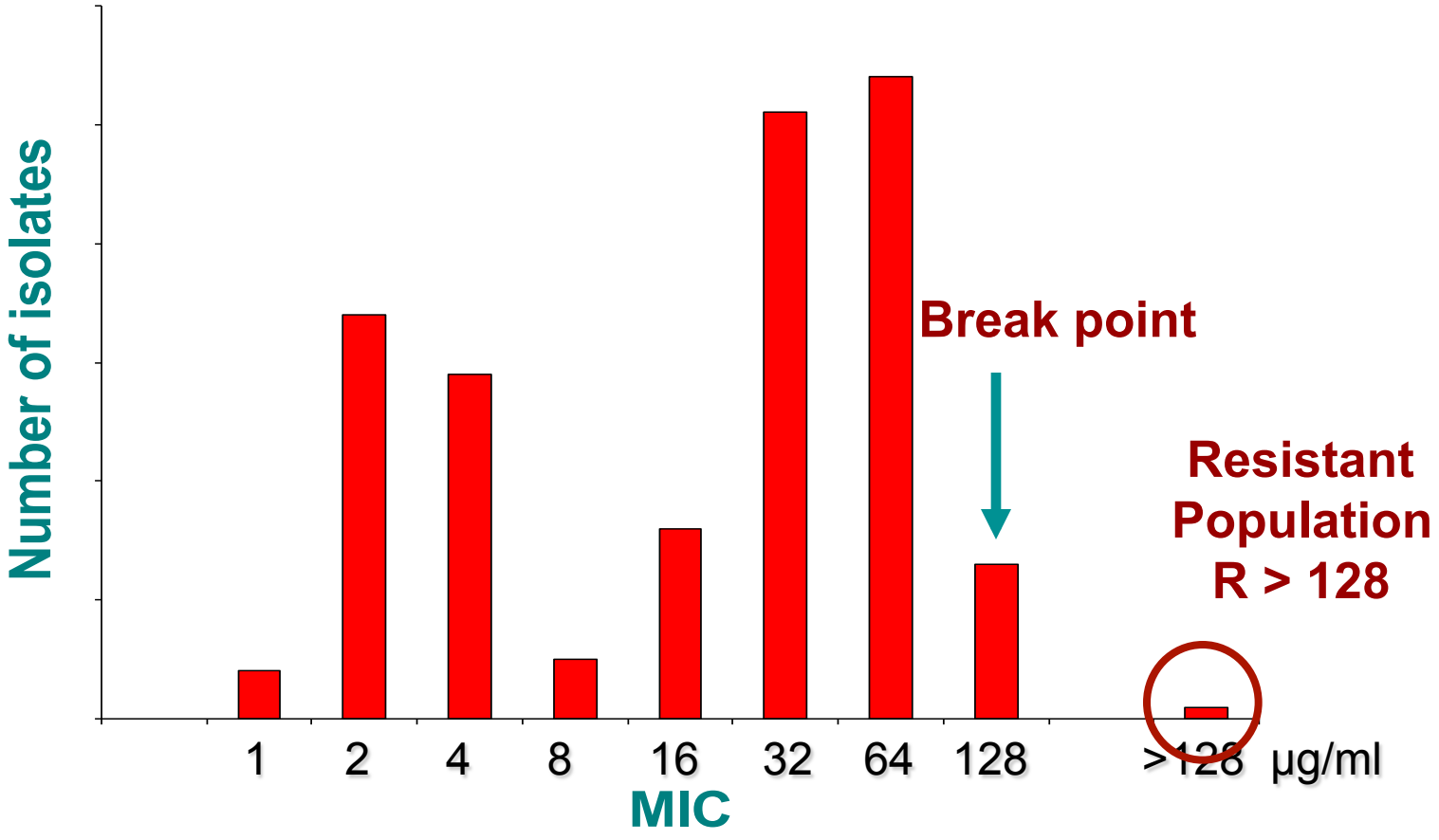
This is called the
“Clinical breakpoint”.

CLSI* is defining the clinical breakpoints.

* Clinical Laboratory Standards Institute)

Population distribution

Drug concentration in infection site: 128 µg/ml



MIC > Breakpoint → Resistant (R > 128)

MIC results….and interpretation.

TABLE 1 Antimicrobial resistance profiles of the two ESBL-producing *Salmonella* serovar Typhi isolates from the Norwegian and Dutc

Antimicrobial class	Antimicrobial(s)	CLSI clinical resistance (*R*) breakpoint (mg/liter)	MIC (mg/liter) for isolate[a]: Strain 1	
Aminocyclitol	Spectinomycin[b]		16	Sensitive
Aminoglycoside	Apramycin[c]		8	Sensitive
	Gentamicin		64	Resistant
	Neomycin[b]		2	Sensitive
	Streptomycin[b]		128	Resistant

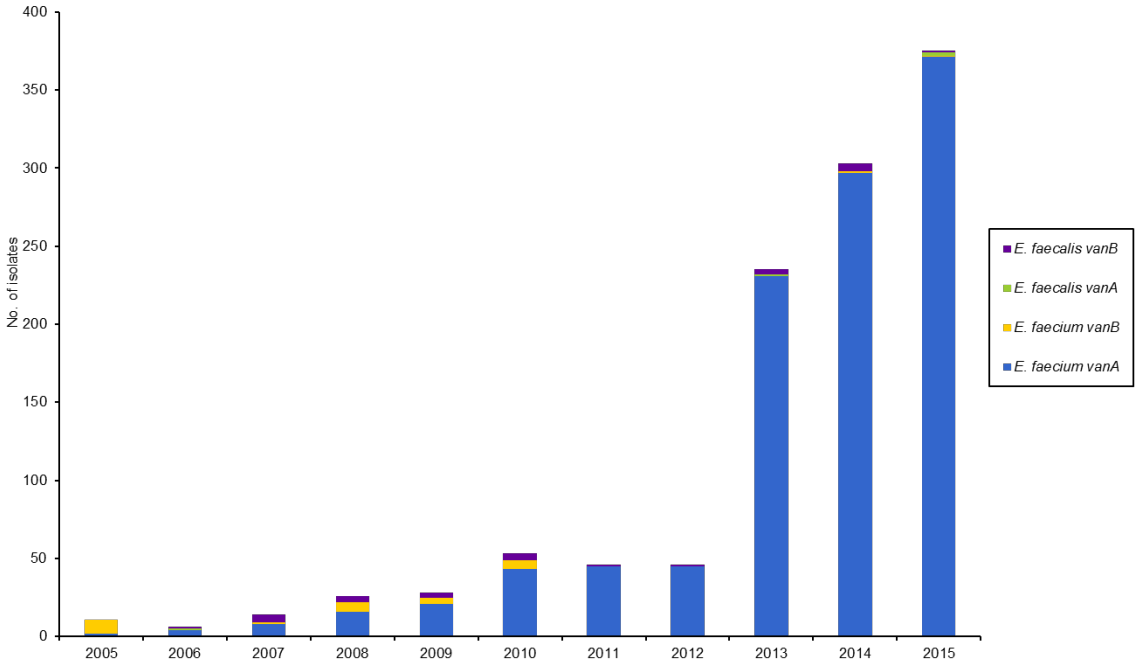
MIC > Breakpoint → Resistant

So when to use what breakpoint?

Microbiological breakpoints:

- Used to monitor development (=surveillance) of resistance in bacterial populations (e.g. on national or global levels)
- Used to detect genes responsible for resistance

Vancomycin resistant enterococci from clinical infections in Denmark



So when to use what breakpoint?

Microbiological breakpoints:

• Used to monitor development (=surveillance) of resistance in bacterial populations (e.g. on national or global levels)

• Used to detect genes responsible for resistance

Clinical breakpoints:

• Used to decide what treatment is suitable for clearing bacterial infections

Consequence/Pitfalls:

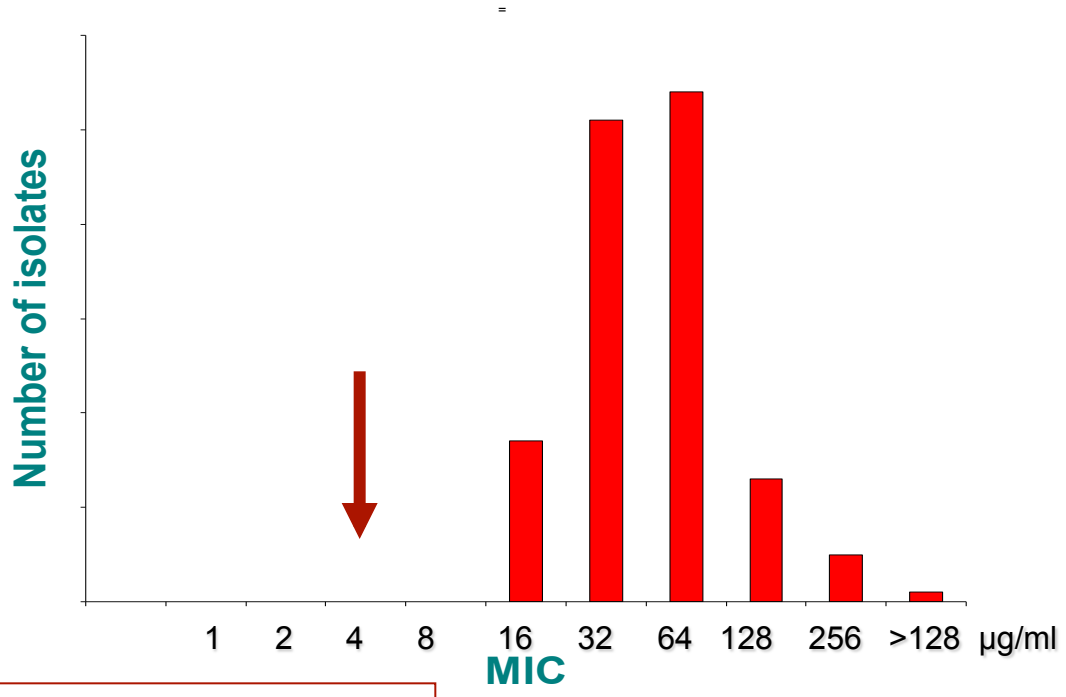
The same drug can have several breakpoints.

Often different laboratories use different breakpoints. Therefore, the same strain collection can have variable levels of resistant bacteria, if tested in different laboratories.

Resistance to Antimicrobial Drugs

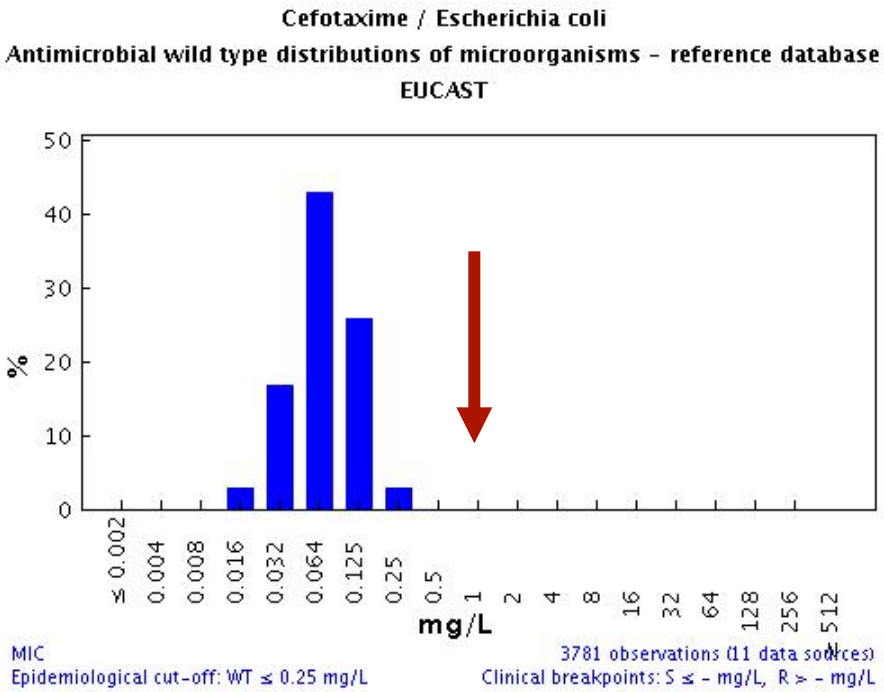
- **The Development of Resistance in Populations**
  - Some pathogens are naturally (*intrinsic*) resistant
    - Gram negative resistant to Glycopeptides and Penicillin G/V
    - Gram positive resistant to polymyxins
  - Resistance by bacteria acquired in two ways
    - New mutations of chromosomal genes
    - Acquisition of resistance genes e.g. on R-plasmids or transposons via transformation, transduction, and conjugation

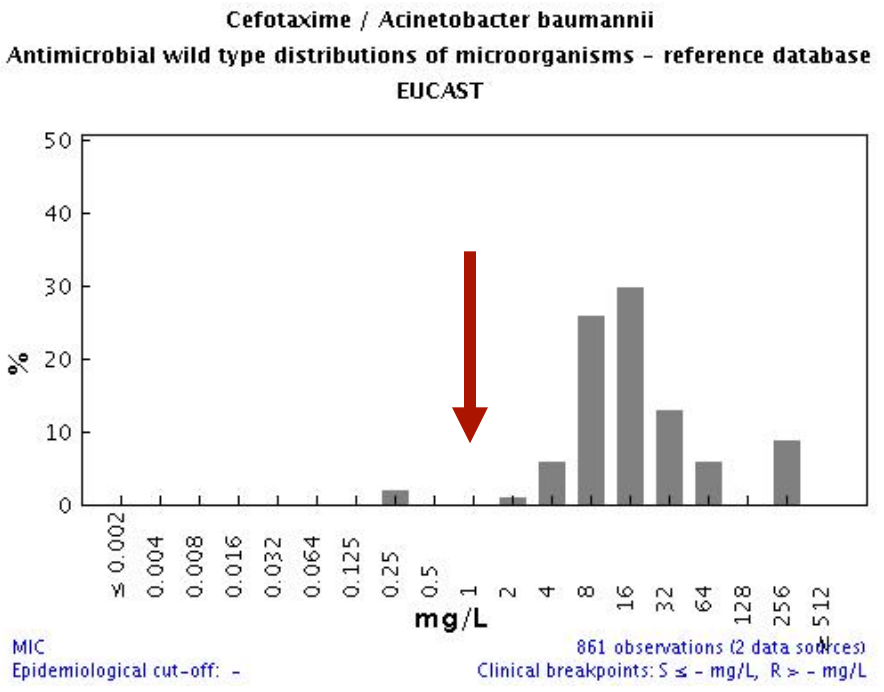
If a complete bacterial species can't be killed by a certain antimicrobial agent in therapy, it is said to be **intrinsic resistant**



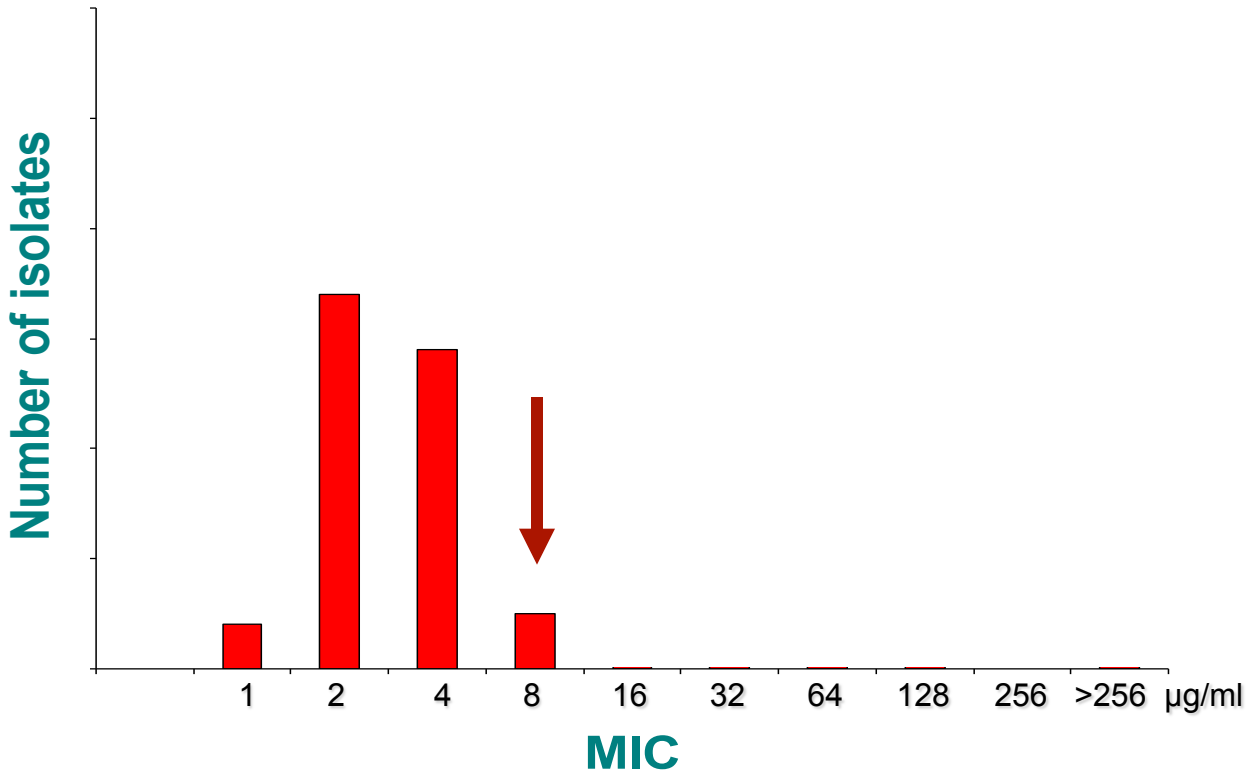
Drug concentration in infection site: 4 µg/ml

Cefotaxime susceptibility in *E. coli* and *Acinetobacter baumannii*





If a only a subset of a bacterial species can be killed by a certain antimicrobial agent in therapy, it has most likely **acquired resistance**.



Multi-, Extensively-, panresistance?

The general definition:

- Multi-resistance is resistance to ≥3 different classes
- Extensively drug-resistance is resistance to all common classes
- Pan resistance is resistance to all drug classes.

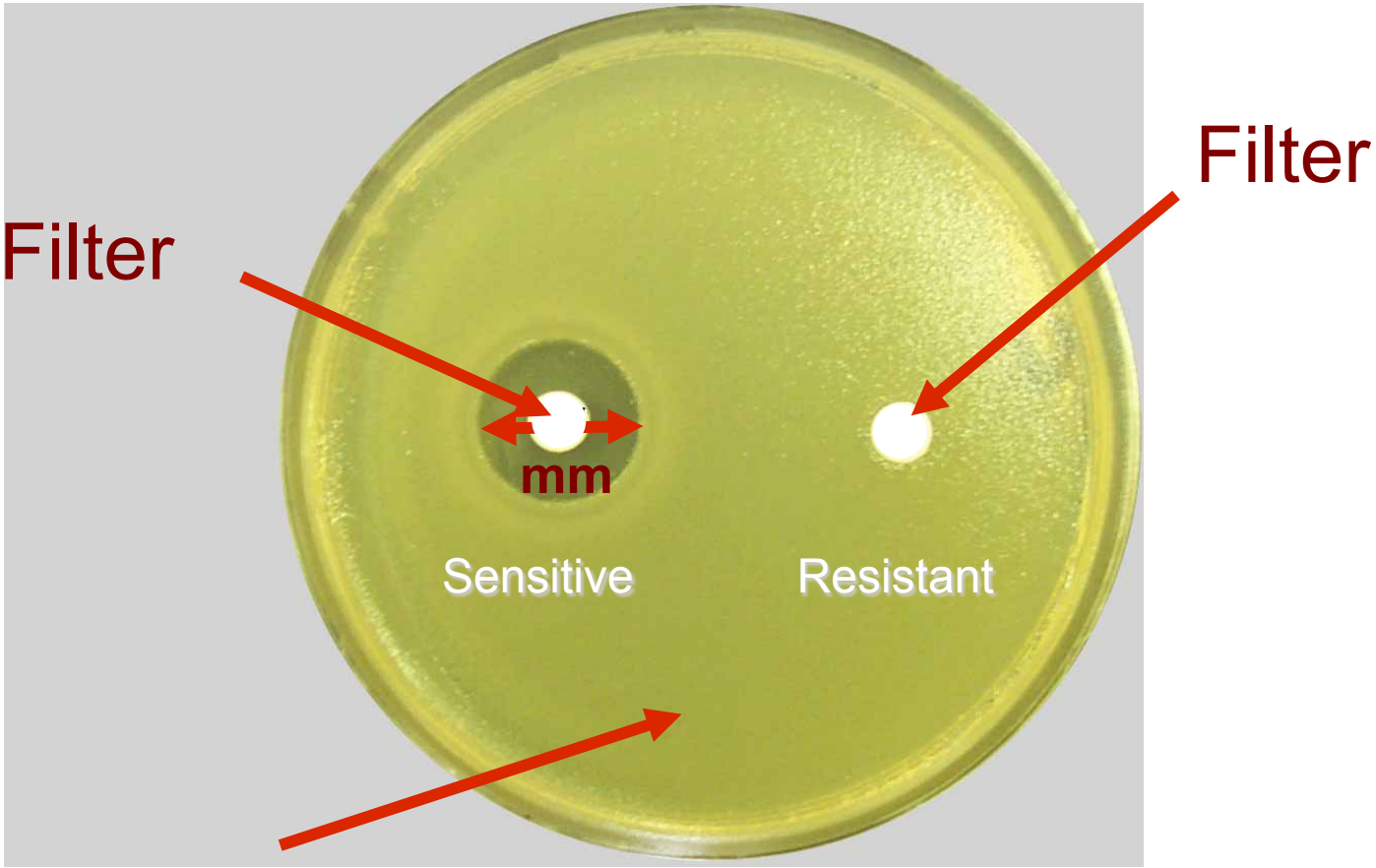
How do we measure antimicrobial susceptibility?

Phenotypic methods

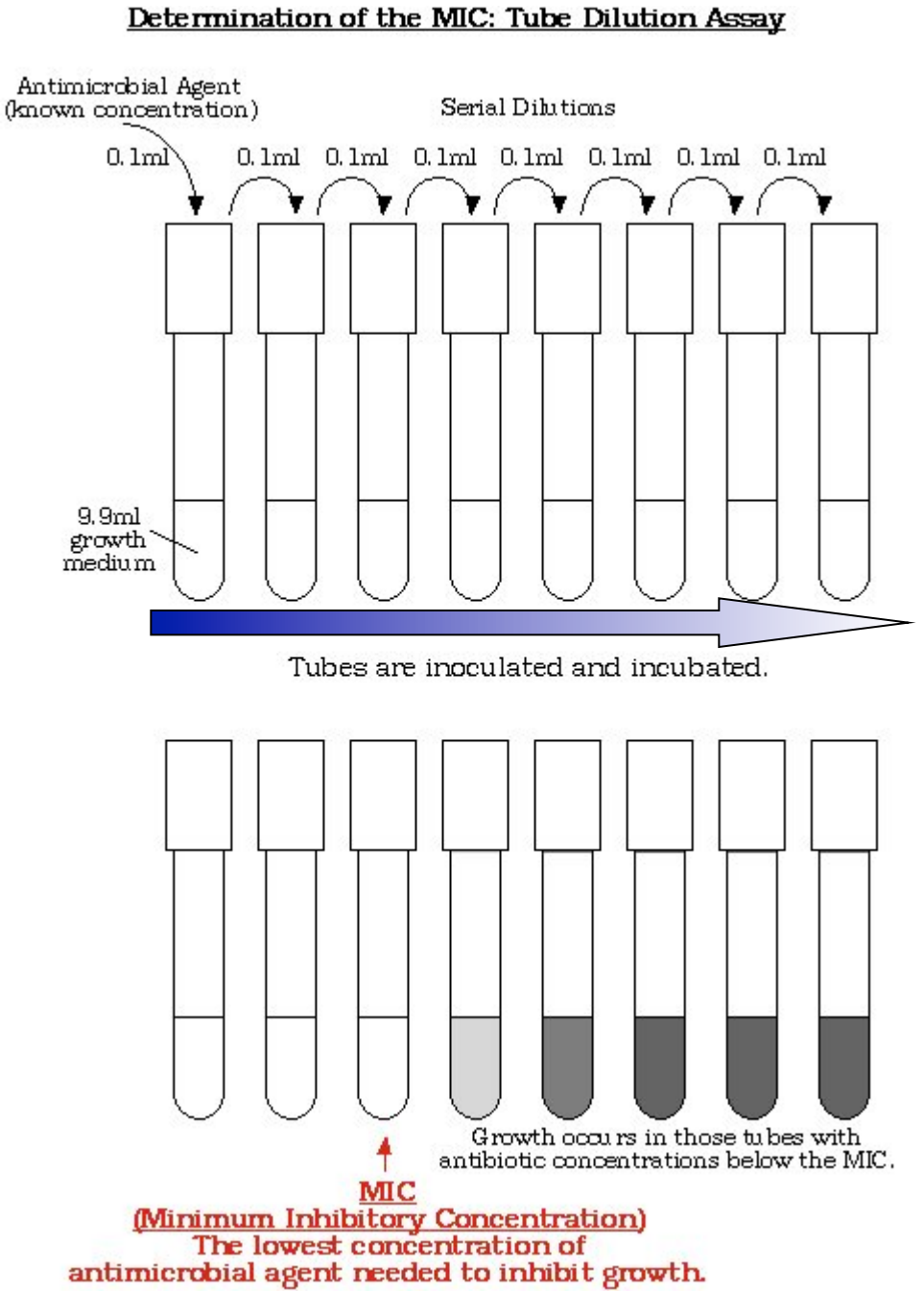
- **Agar diffusion method**
  - Disk (tablet) methods
  - E-test (quantitative)

- **Dilution methods**
  - Liquid media (quantitative)
  - Solid media (quantitative)

Disc diffusion



Dilution method

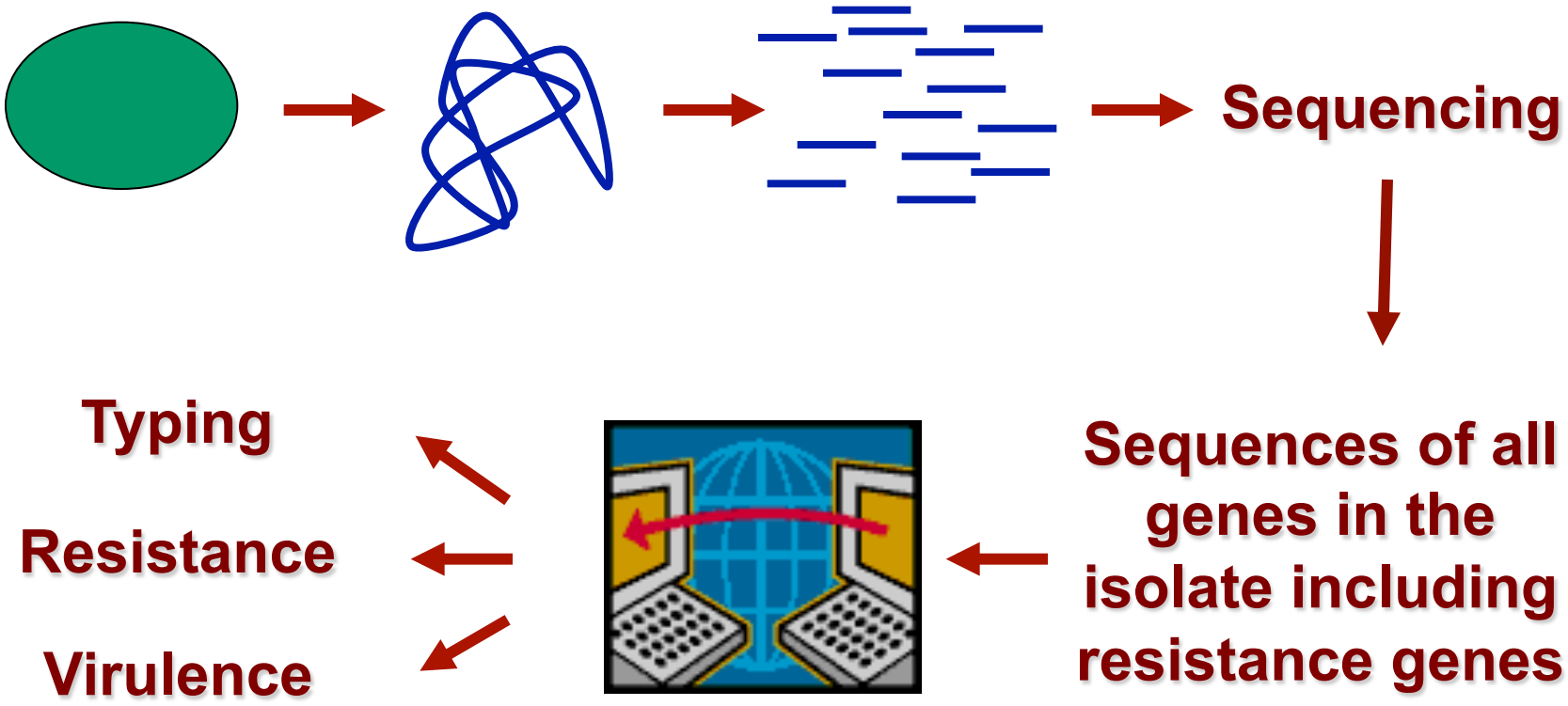


How do we detect antimicrobial susceptibility?

Genotypic methods

- PCR for resistance genes
- DNA arrays
- Whole genome sequencing

Whole genome sequencing



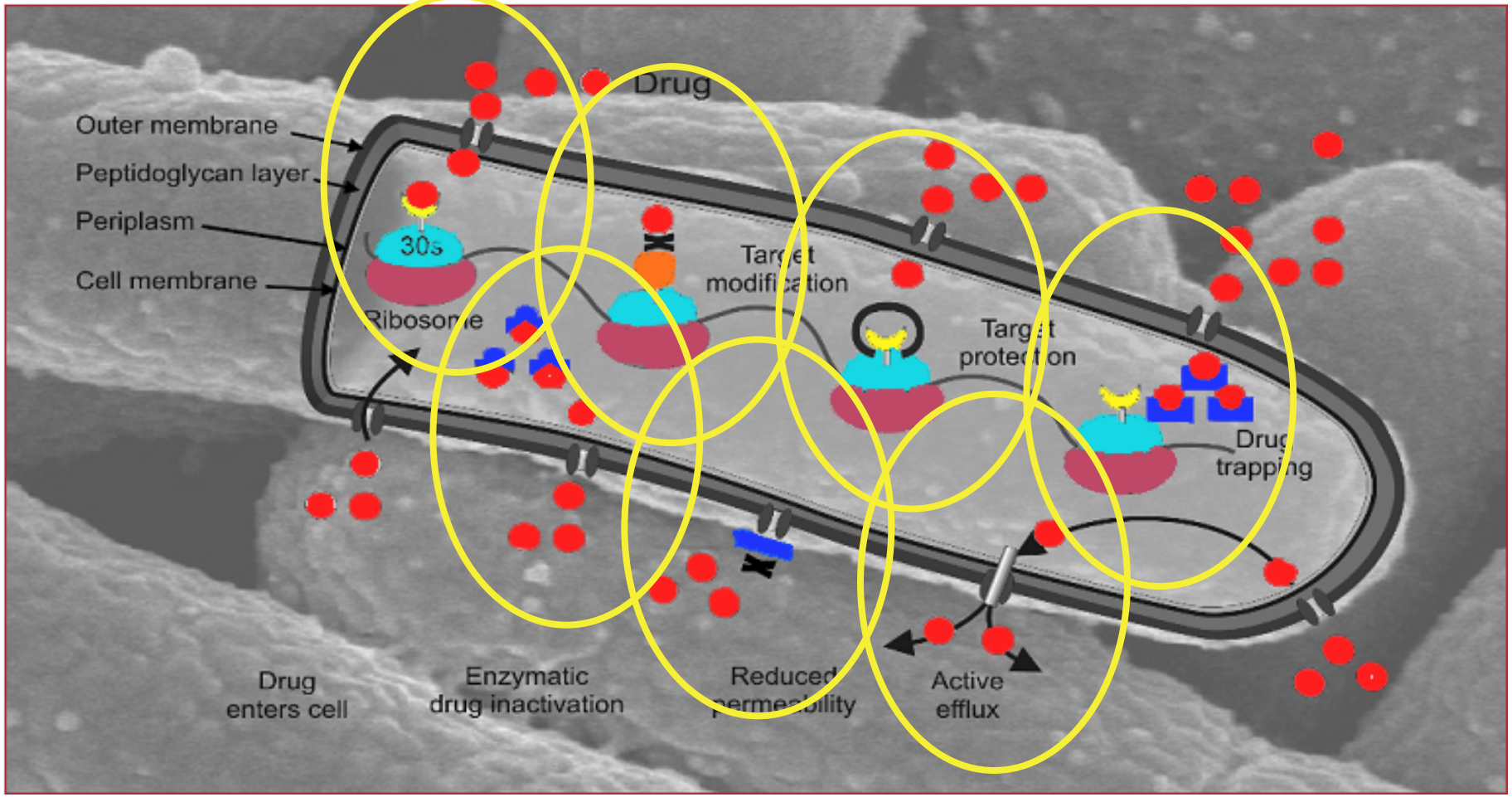
BREAK

Mechanisms of acquired antimicrobial resistance?

Resistance to Antimicrobial Agents

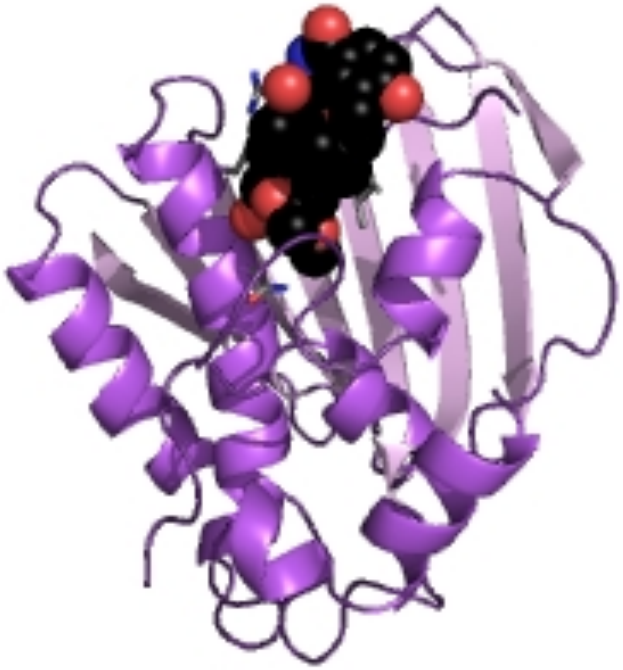
- **The Development of Resistance in Populations**
  - Some pathogens are naturally (*intrinsic*) resistant
    - Gram negative resistant to Glycopeptides and Penicillin G/V
    - Gram positive resistant to polymyxins
  - Resistance by bacteria acquired in two ways
    - New mutations of chromosomal genes
    - Acquisition of resistance genes e.g. on R-plasmids or transposons via transformation, transduction, and conjugation

Antibiotics: Modes of resistance



Genetic variations/Point mutations

DNA gyrase –quinolone resistance



Genetic variations/Point mutations

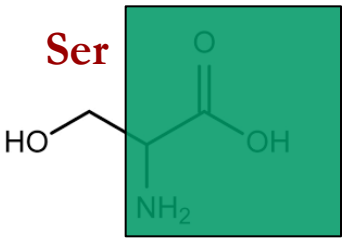
DNA gyrase – 1 mutation = quinolone resistance

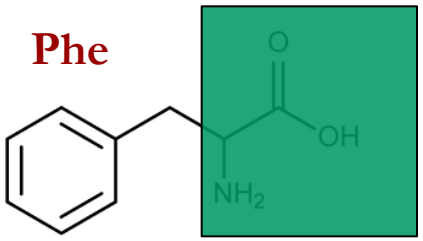
		110	120	130	140	150
Nal^S	101	TGACGTAATC	GGTAAATACC	ATCCCCACGG	CGATTCCGCA	GTGTATGACA
Nal^R MUT83A	101	TGACGTAATC	GGTAAATACC	ATCCCCACGG	CGATTACGCA	GTGTATGACA
Nal^R MUT83T	101	TGACGTAATC	GGTAAATACC	ATCCCCACGG	CGATTTCGCA	GTGTATGACA

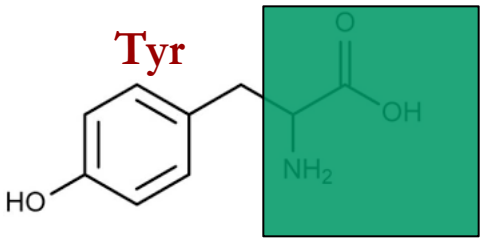
Codon 83: TCC → Ser

TTC → Phe

TAC → Tyr

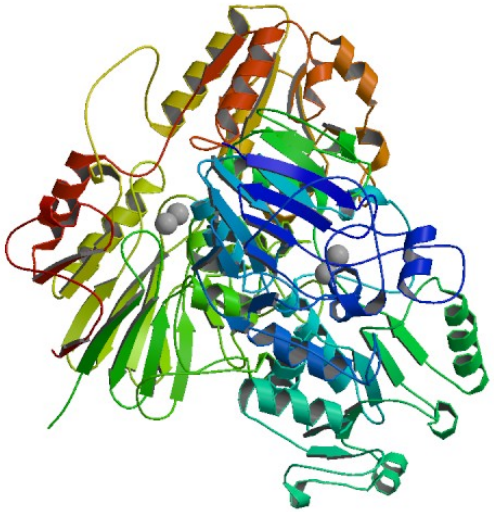






Beta-lactamases

An example



What are they?

Proteins degrading Beta-lactam’s

The Beta-lactam antibiotics

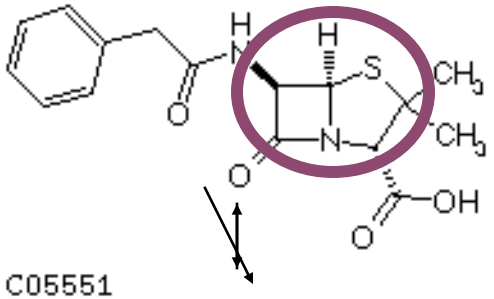
- Isolated from *Penicillium spp. or Cephalosporium spp.*
- App. 50 % of the antibiotics used worldwide
- Is now being produced semi-synthetically
- Kills growing cells by interfering with the cell-wall synthesis

Three important sub-classes of the beta-lactams

- Penicillins
- Cephalosporins
- Carbapenems

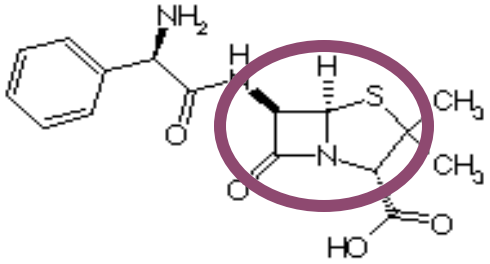
Penicillins

Penicillin G



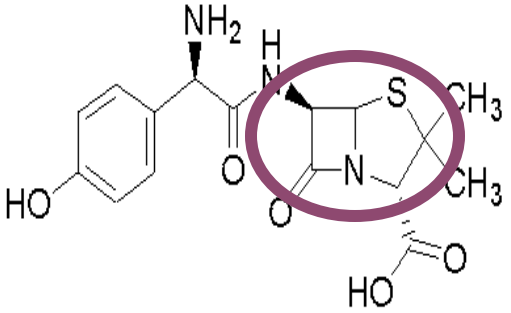
C05551

Ampicillin (AMP)



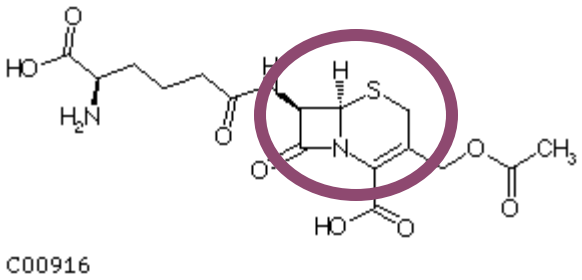
C06574

Amoxicillin

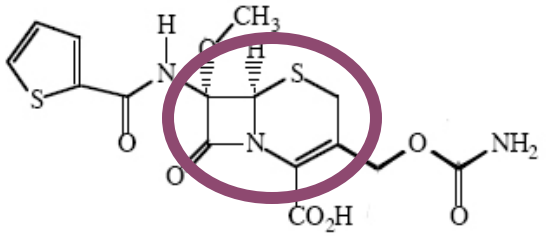


Cephalosporin's

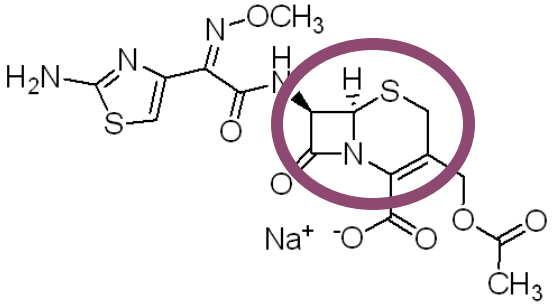
Cephalosporin C
(1. gen. Cephalosporin)



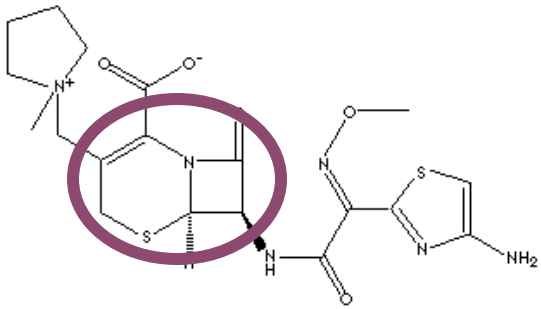
Cefoxitin (FOX)
(2. gen. cephamycin)



Cefotaxime (CTX)
(3. gen. Cephalosporin)

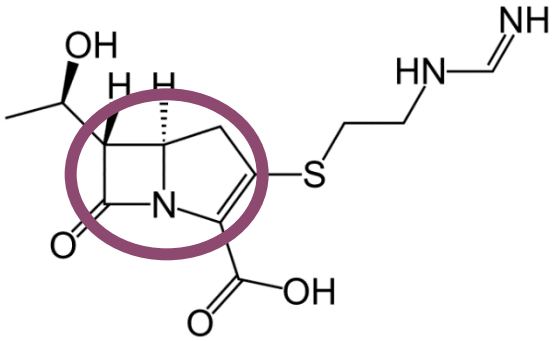


Cefepime (FEB)
(**4**. gen. cephalosporin)

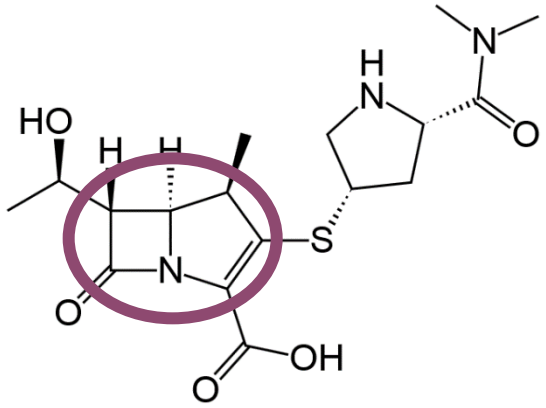


Carbapenems

Imipenem (IMI)



Meropenem (MERO)



Narrow spectrum vs. Extended spectrum Beta-lactam's

Narrow and moderate spectrum beta-lactams

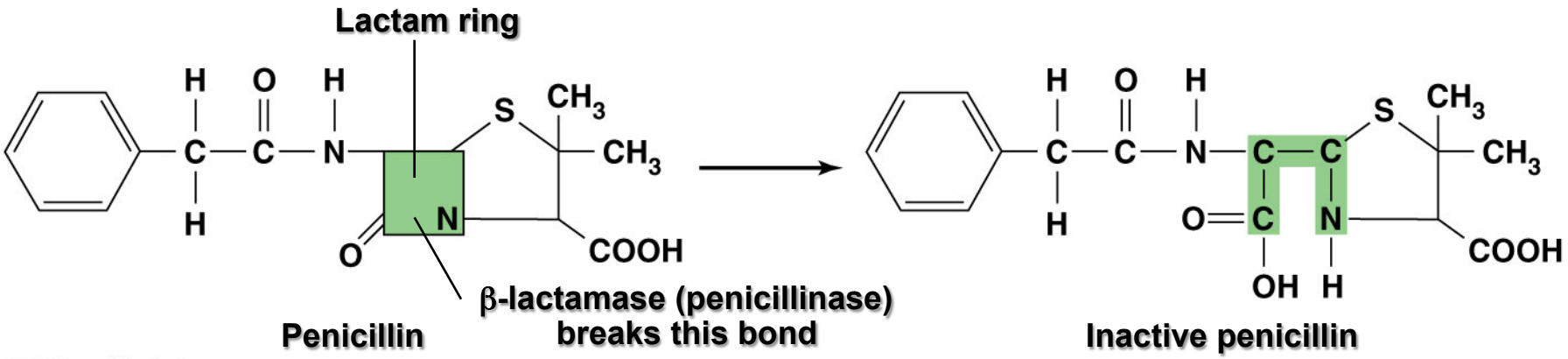
- Penicillin G and V (PEN)
- Methicillin (MET) → ***mecA* in *S. aureus***
- amoxicillin (AMOX) and ampicillin (AMP)
- Cephalotin (CEP)

Enzymes, which can degrade these drugs are called penicillinases or ampicillinases.

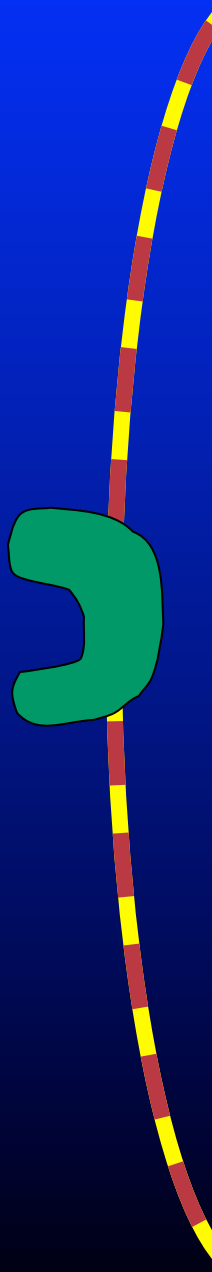
Broad and Extended spectrum beta-lactams

- Cefoxitin (FOX)
- Cefotaxime (CTX) and Ceftazidime (CAZ)
- Cefepime (FEB)
- Imipenem (IMI)

Enzymes, which can degrade these drugs are called cephalosporinases or carbapenemases.



Beta-lactam



Ampicillin

Penicillin binding protein (PBP)

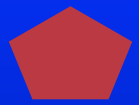
Beta-lactamase

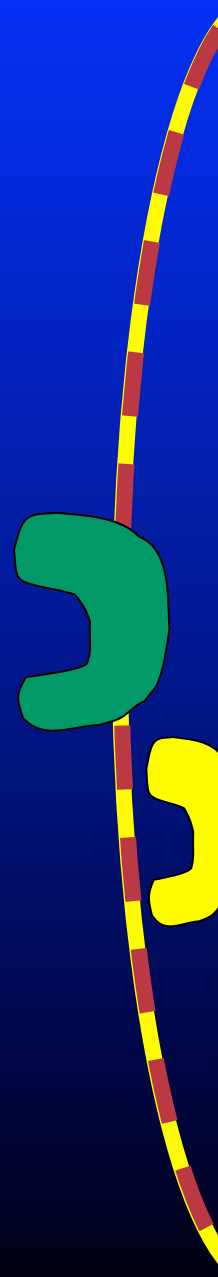
Ampicillin

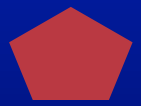
Penicillin binding protein (PBP)

Ampicillinase (TEM-1 or AmpC)

Extended-spectrum Beta-lactams





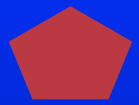


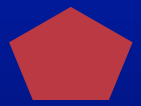
Cephalosporins (AXO)

Penicillin binding protein (PBP)

Penicillinase (TEM-1 or AmpC)

Beta-lactamase





Cephalosporins (AXO)

Penicillin binding protein (PBP)

Extended-Spectrum Beta-Lactamase (SHV-12, CTX-M..)

Plasmidic AmpC's	ESBL	MBL
CMY	**TEM***	**IMP**
ACC	**SHV***	**VIM**
DHA	**CTX-M**	**KPC**
FOX	OXA*	SPM
BIL	VEB	GIM
MIR	PER	
ACT	CME	
KLU	SFO	
	FEC	
	GES	

Genes in red indicate most prevalent types!

* Only some variants are cephalosporinases

Example – Resfinder

Center for Genomic Epidemiology

Home | Services | Instructions | Output | Overview of genes | Article abstract

ResFinder 2.0 (Acquired antimicrobial resistance gene finder)

ResFinder identifies acquired antimicrobial resistance genes in total or partial sequenced isolates of bacteria.
Fasta file with test sequence: Test_sequence
NOTE: Currently ResFinder focuses on acquired genes and do therefore not find chromosomal mutations (NAL, FUS, high-level CIP, RIF resistance, etc.)

View the version history of this server.

Browse | Remove | Clear

Uploads

Total files: 0 (N/A).

Proble

Select Antimicrobial configuration
Select multiple items, with Ctrl-Click (or Cmd-Click on Mac)

All
Aminoglycoside
Beta-lactamase
Fluoroquinolone
Fosfomycin
Fusidic Acid

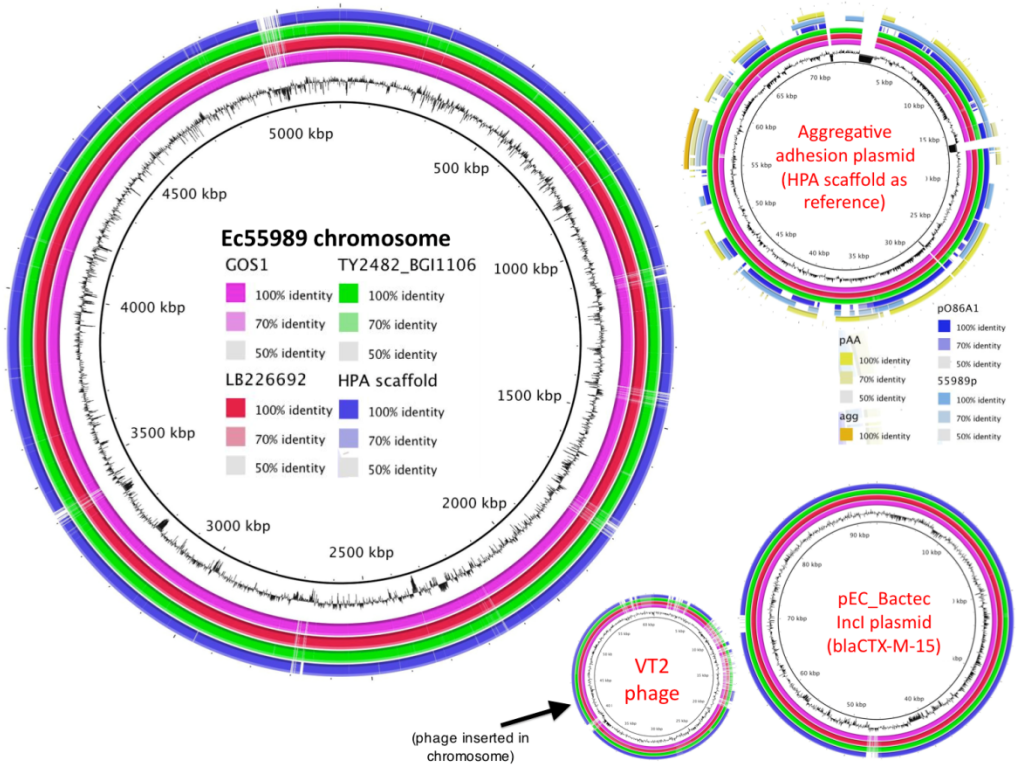
Select threshold for %ID
98 %

Select minimum length
Length a gene in the genome at least has to cover of the length of the resistance gene in the d
60 %

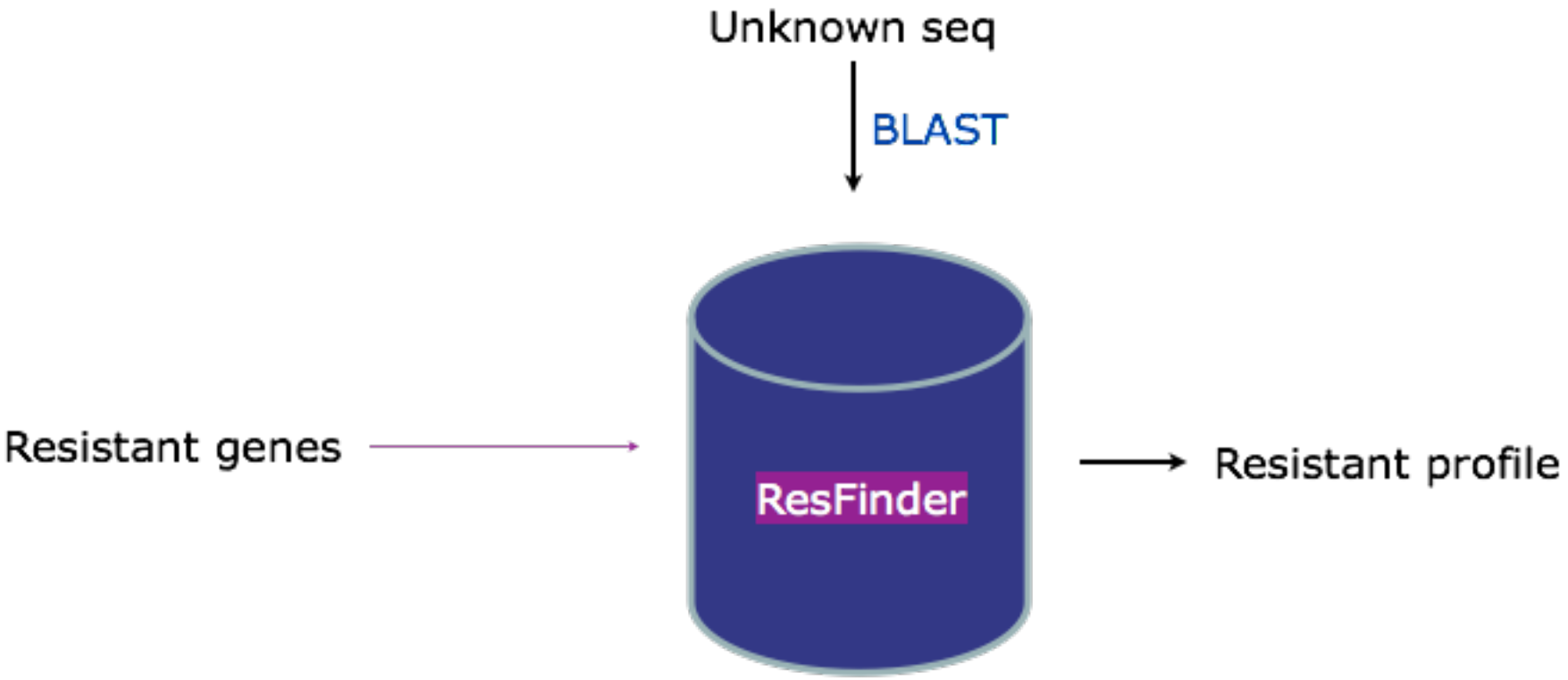
Select type of your reads
Assembled Genome/Contigs*

Submit | Clear fields

Confidentiality:
The sequences are kept confidential and will be deleted after 48 hours.



Resfinder – How does it work?



Aminoglycoside						
Resistance gene	%Identity	HSP/Query length	Contig	Position in contig	Predicted phenotype	Accession number
strA	100.00	804 / 804	TY-2482_chromosome	3130176..3130979	Aminoglycoside resistance Alternate name; aph(3")-Ib	AF321551
strB	100.00	837 / 837	TY-2482_chromosome	3130979..3131815	Aminoglycoside resistance Alternate name; aph(6)-Id	M96392

Beta-lactam						
Resistance gene	%Identity	HSP/Query length	Contig	Position in contig	Predicted phenotype	Accession number
blaCTX-M-15	100.00	876 / 876	TY-2482_pTY1	24045..24920	Beta-lactam resistance Alternate name; UOE-1	DQ302097
blaTEM-1	100.00	861 / 861	TY-2482_pTY1	27742..28602	Beta-lactam resistance Alternate name; RblaTEM-1	JF910132

Sulphonamide						
Resistance gene	%Identity	HSP/Query length	Contig	Position in contig	Predicted phenotype	Accession number
sul1	100.00	761 / 840	TY-2482_chromosome	3123387..3124147	Sulphonemide resistance	AY224185
sul2	100.00	816 / 816	TY-2482_chromosome	3129300..3130115	Sulphonamide resistance	HQ840942
sul3	99.74	759 / 852	TY-2482_chromosome	3123389..3124147	Sulphonamide resistance	AB281182

Tetracycline						
Resistance gene	%Identity	HSP/Query length	Contig	Position in contig	Predicted phenotype	Accession number
tet(A)	100.00	1200 / 1200	TY-2482_chromosome	3142018..3143217	Tetracycline resistance	AJ517790

Trimethoprim						
Resistance gene	%Identity	HSP/Query length	Contig	Position in contig	Predicted phenotype	Accession number
dfrA17	91.14	474 / 474	TY-2482_chromosome	3122343..3122816	Trimethoprim resistance	FJ460238
dfrA7	100.00	474 / 474	TY-2482_chromosome	3122343..3122816	Trimethoprim resistance	JF806498

But the servers are so slow….

You can install our tools on your own computer

https://bitbucket.org/genomicepidemiology/cge-tools-docker/src

By using the Docker system you can easily install and run our programs locally