What is antimicrobial resistance II?

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

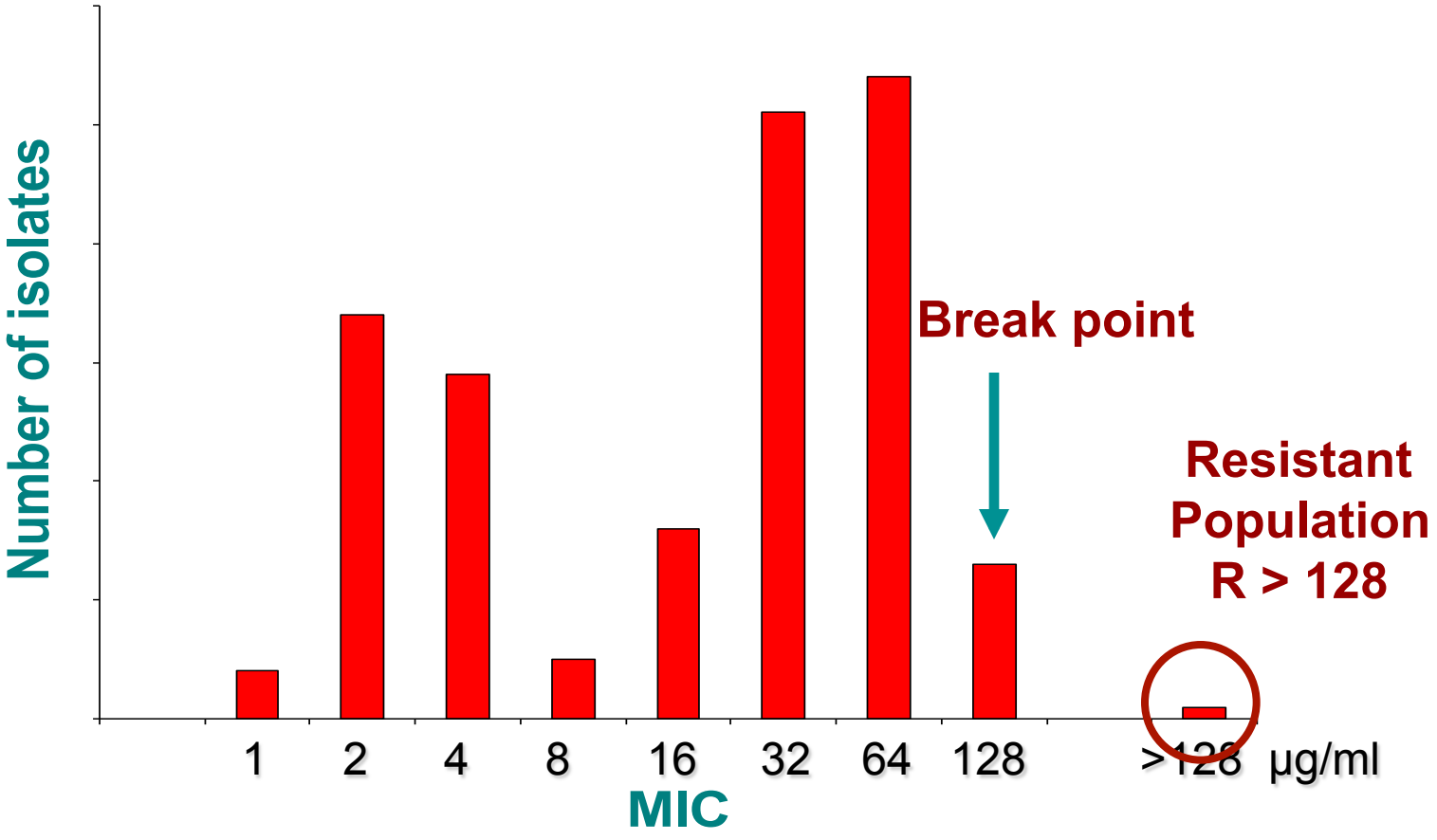
This is called the
“Clinical breakpoint”.

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

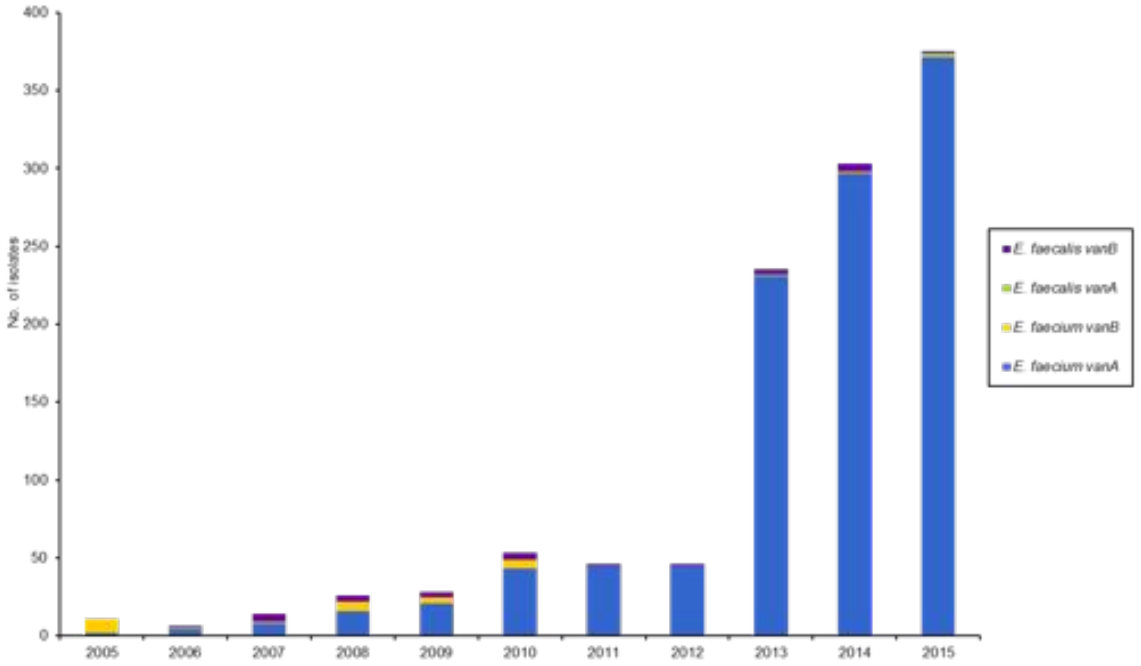
MIC > Breakpoint → Resistant

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:

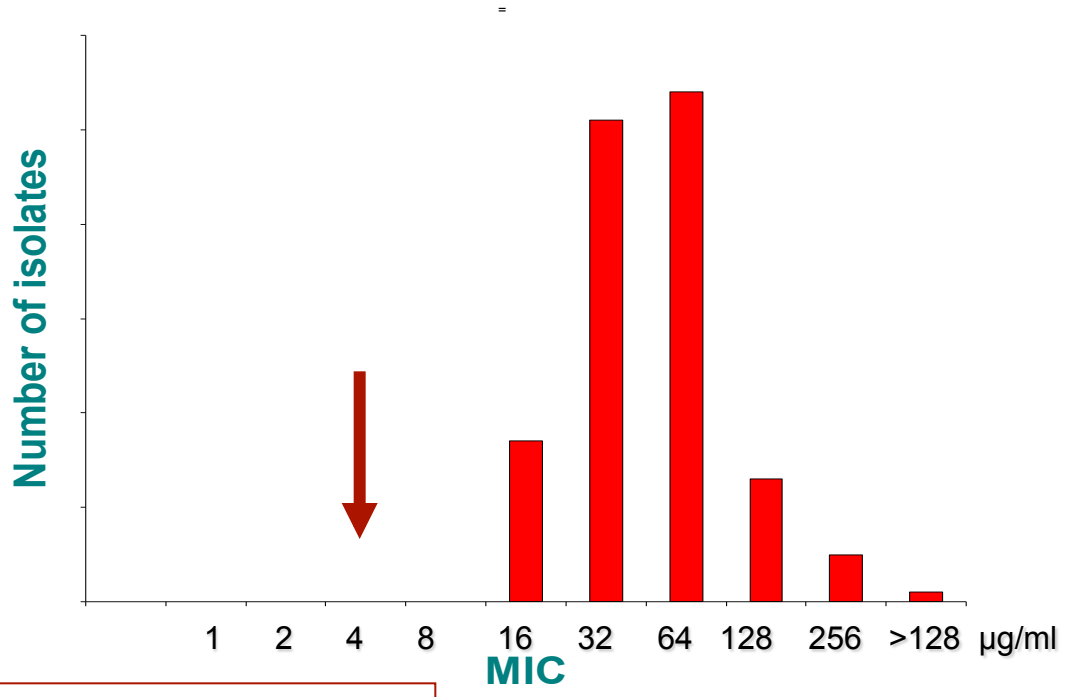
The same drug can have several breakpoints.

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

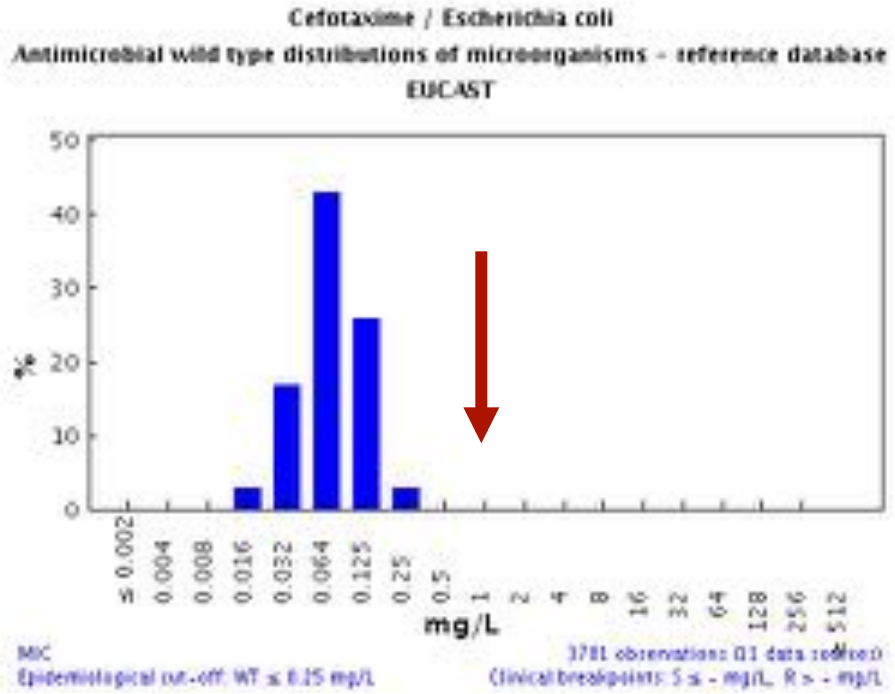
- **The Development of Resistance in Populations**
  - Some pathogens are naturally (*intrinsic*) resistant
    - Gram negative resistant to Glycopeptides and Penicillin G/V
    - Gram positive resistant to polymyxins
  - Resistance by bacteria acquired in two ways
    - New mutations of chromosomal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

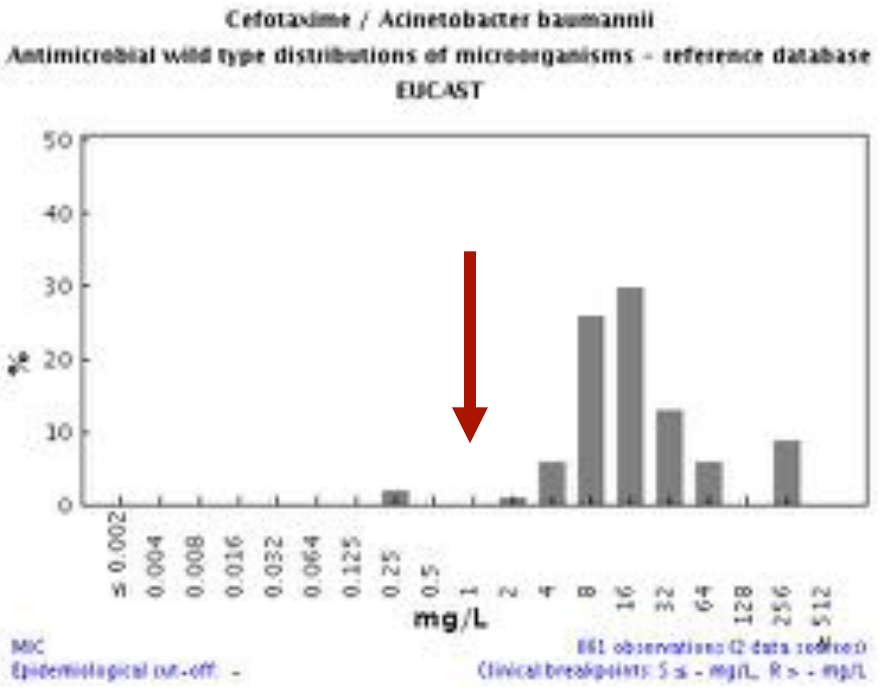
If a complete bacterial species can't be killed by a certain antimicrobial agent in therapy, it is said to be **intrinsic resistant**



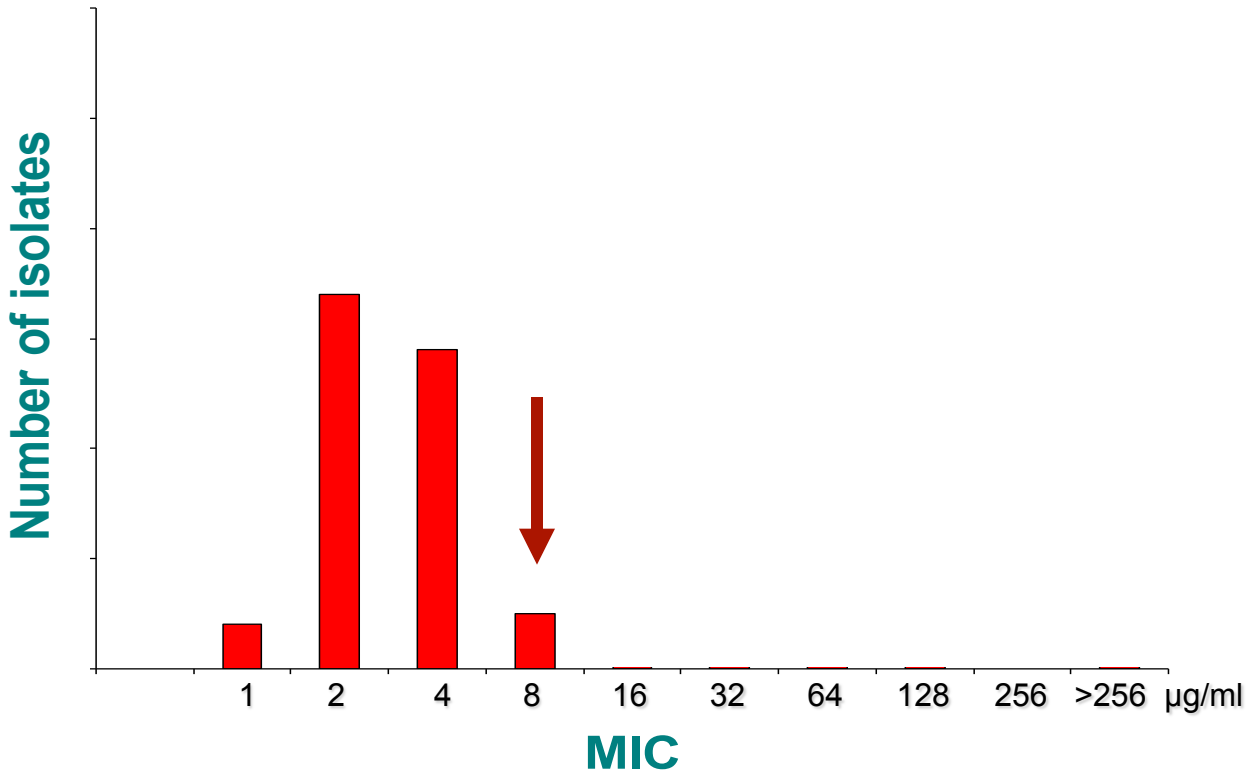
Drug concentration in infection site: 4 µg/ml

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.

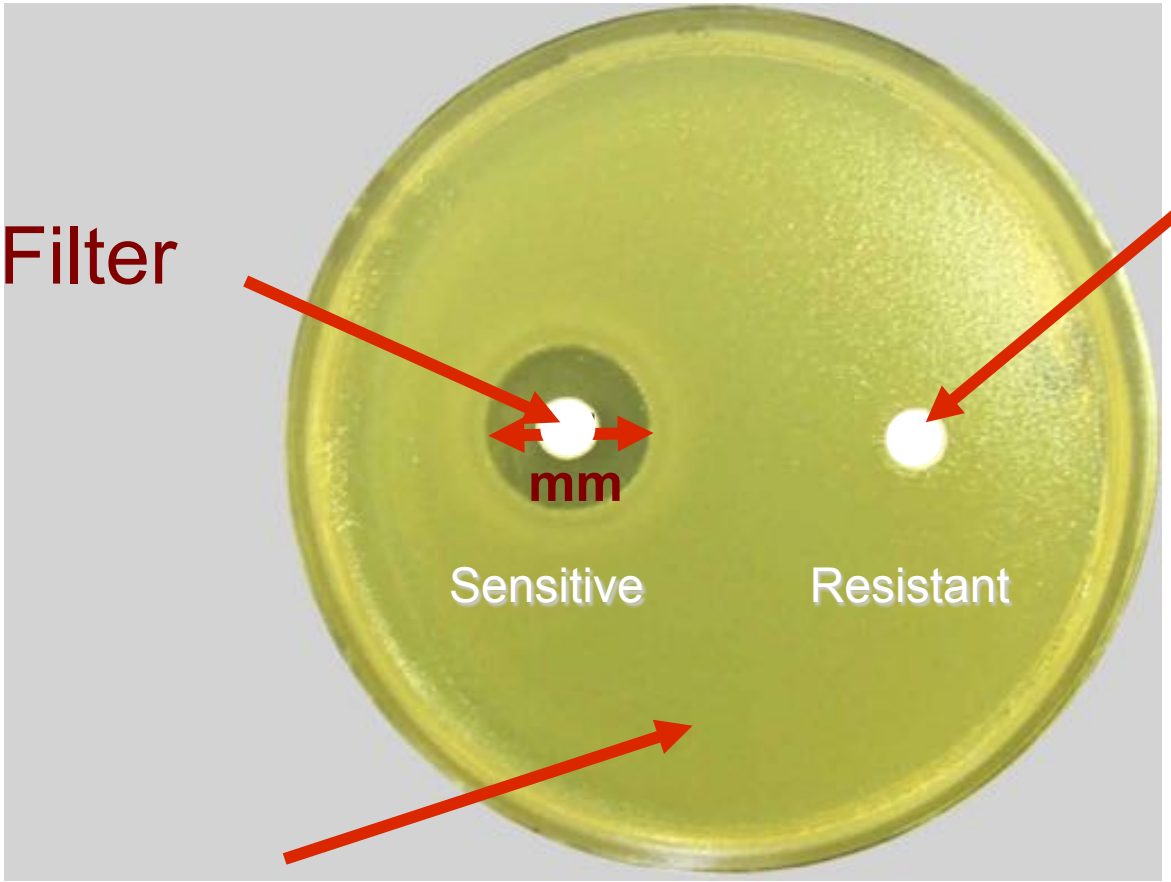
How do we measure antimicrobial susceptibility?

Phenotypic methods

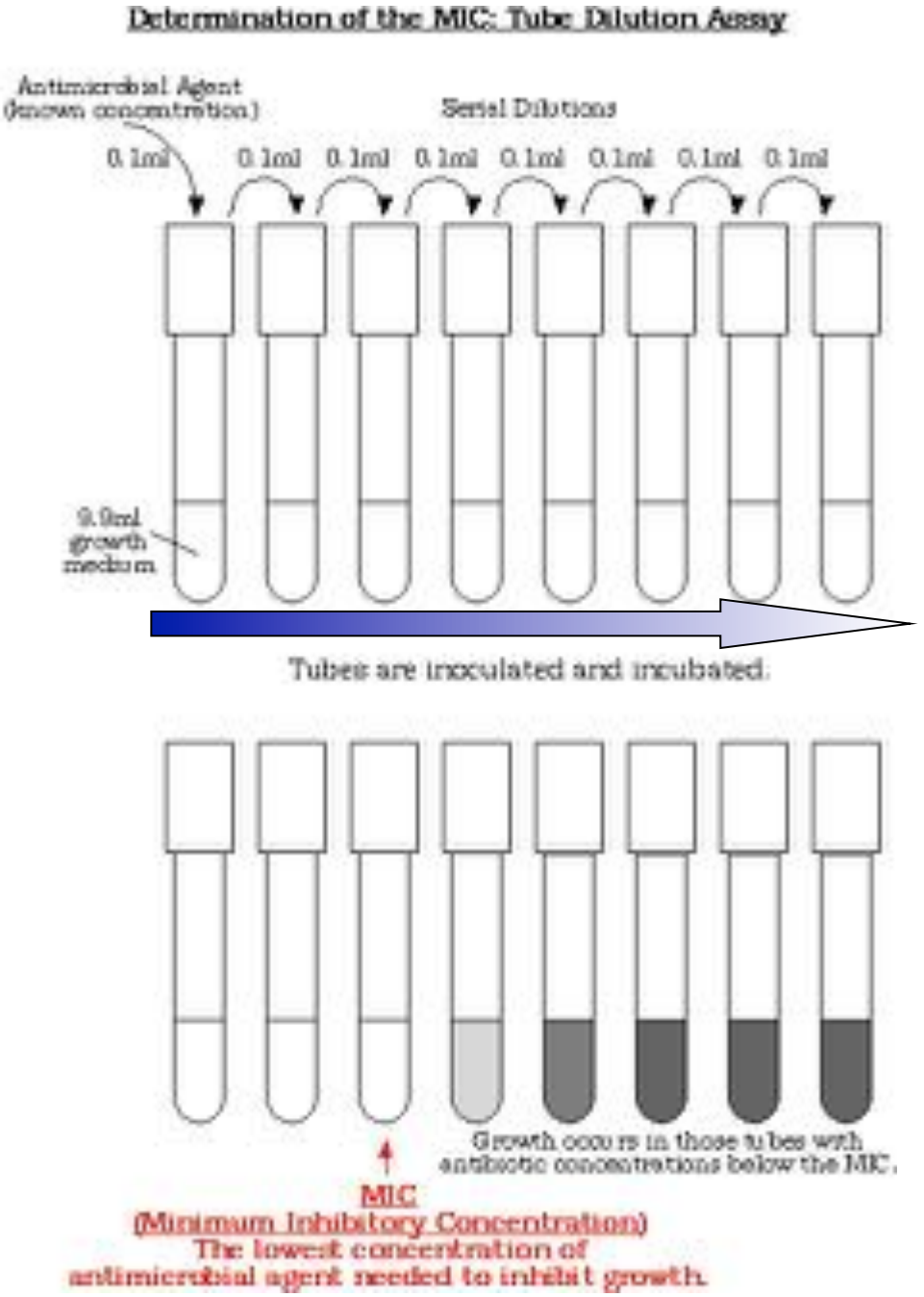
- **Agar diffusion method**
  - Disk (tablet) methods
  - E-test (quantitative)

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

Disc diffusion



Dilution method

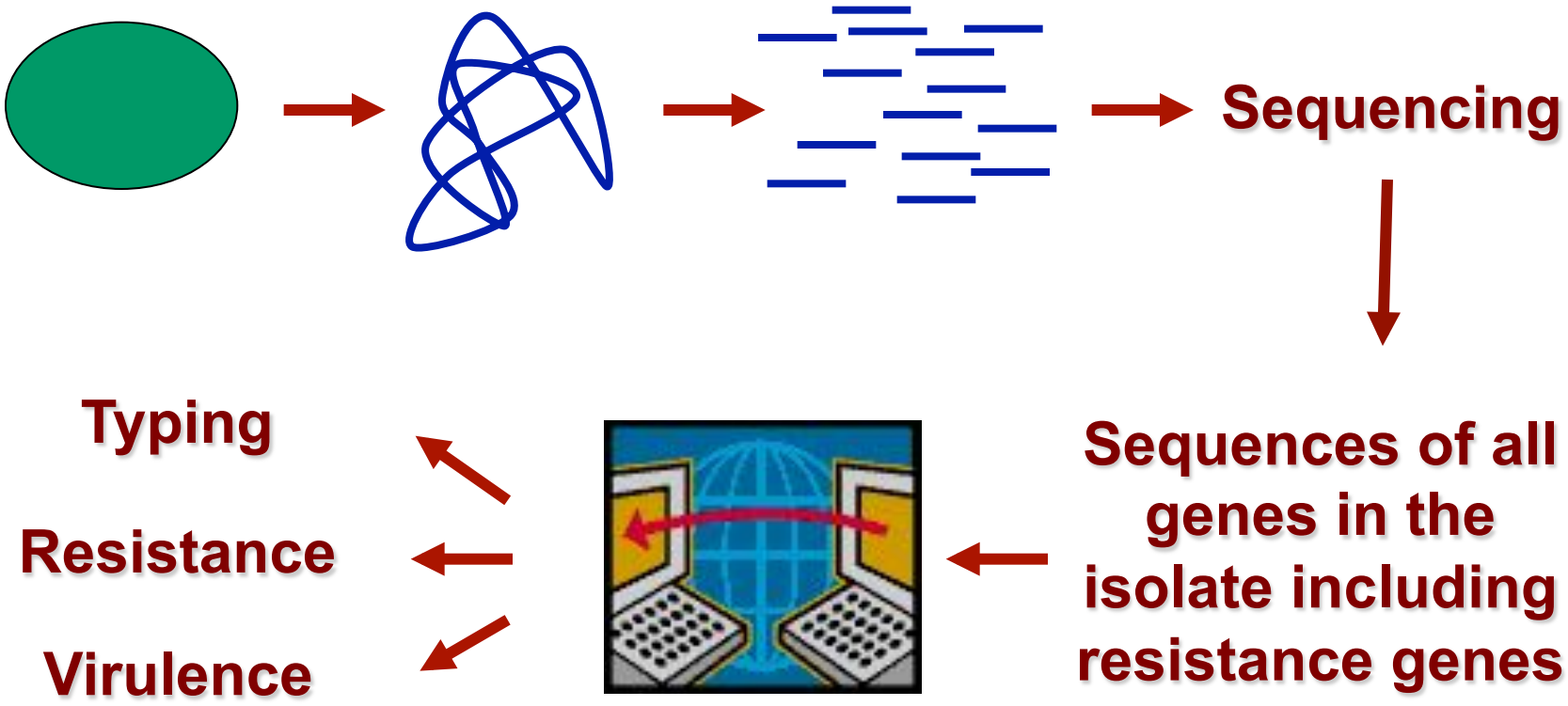


How do we detect antimicrobial susceptibility?

Genotypic methods

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

Whole genome sequencing



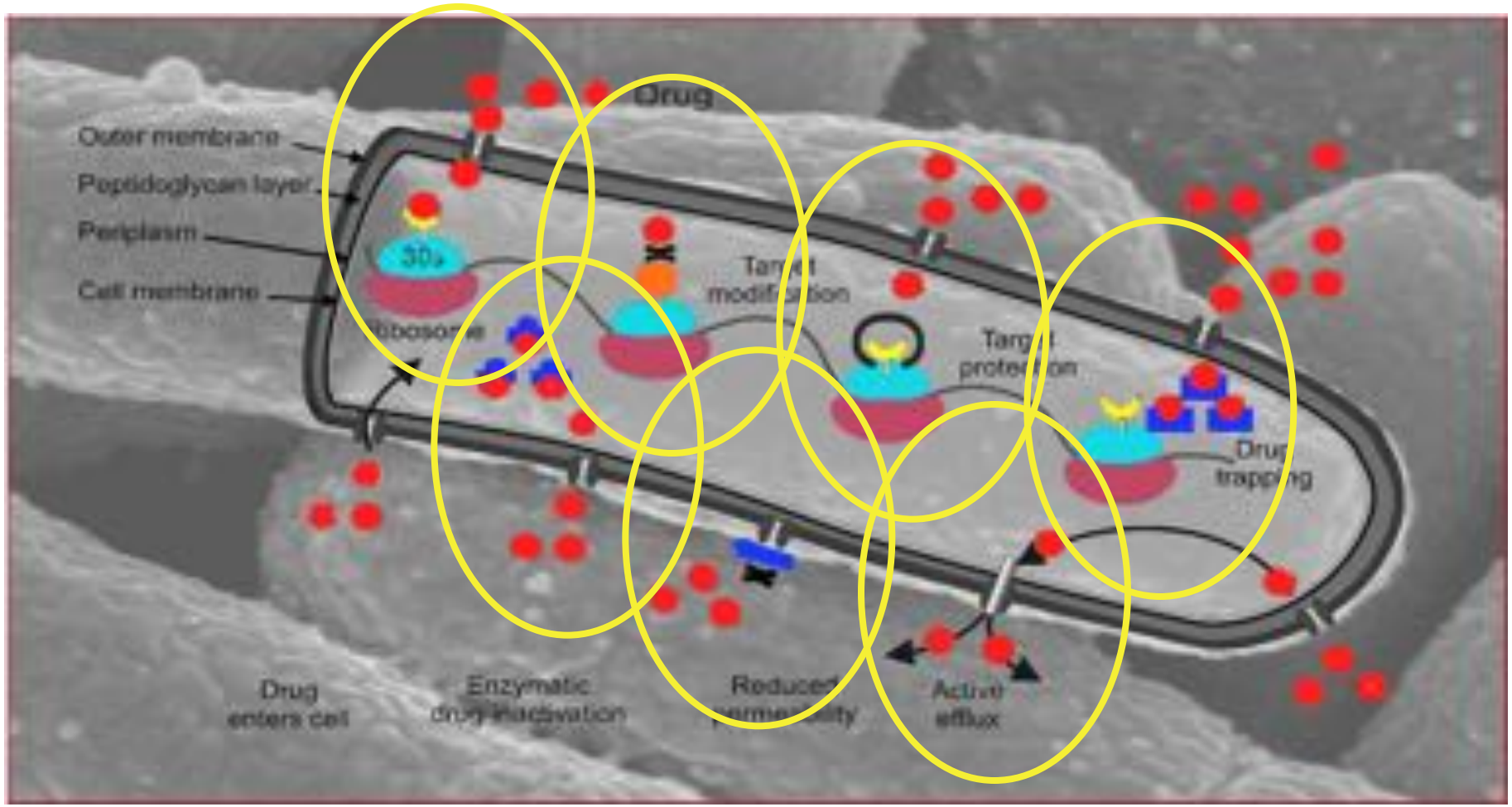
BREAK

Mechanisms of acquired antimicrobial resistance?

Resistance to Antimicrobial Agents

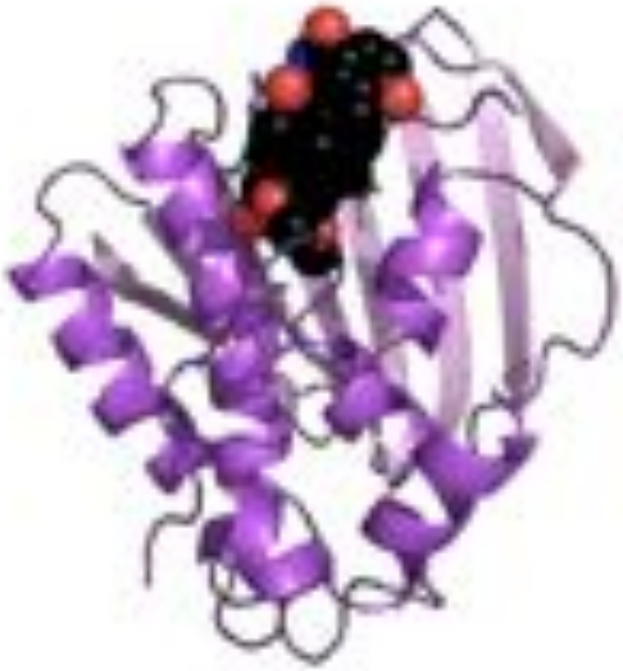
- **The Development of Resistance in Populations**
  - Some pathogens are naturally (*intrinsic*) resistant
    - Gram negative resistant to Glycopeptides and Penicillin G/V
    - Gram positive resistant to polymyxins
  - Resistance by bacteria acquired in two ways
    - New mutations of chromosomal 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

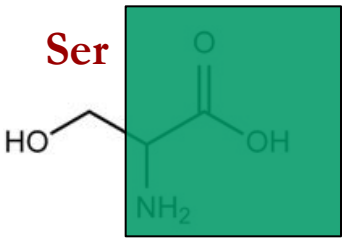
DNA gyrase – 1 mutation = quinolone resistance

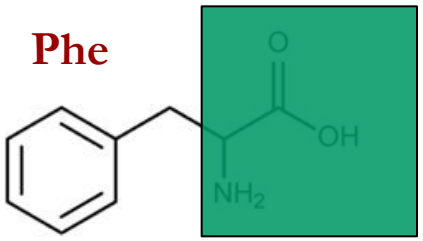
		110	120	130	140	150
Nal^S	101	TGACGTAATC	GGTAAATACC	ATCCCCACGG	CGATTCCGCA	GTGTATGACA
Nal^R MUT83A	101	TGACGTAATC	GGTAAATACC	ATCCCCACGG	CGATTACGCA	GTGTATGACA
Nal^R MUT83T	101	TGACGTAATC	GGTAAATACC	ATCCCCACGG	CGATTTCGCA	GTGTATGACA

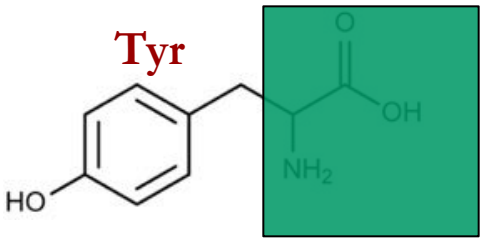
Codon 83: TCC → Ser

TTC → Phe

TAC → Tyr







Beta-lactamases

An example

What are they?

Proteins degrading Beta-lactam’s

The Beta-lactam antibiotics

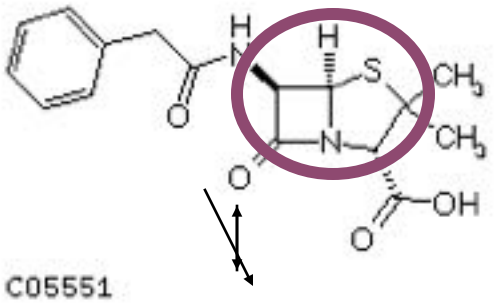
- Isolated from *Penicillium spp. or Cephalosporium spp.*
- App. 50 % of the antibiotics used worldwide
- Is now being produced semi-synthetically
- Kills growing cells by interfering with the cell-wall synthesis

Three important sub-classes of the beta-lactams

- Penicillins
- Cephalosporins
- Carbapenems

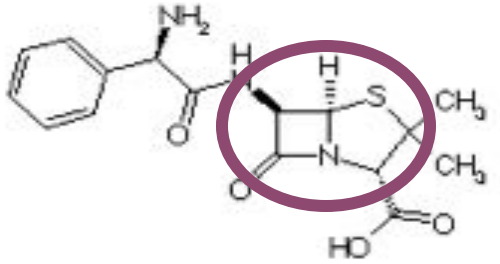
Penicillins

Penicillin G



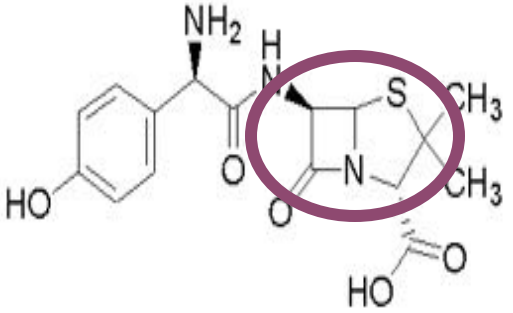
C05551

Ampicillin (AMP)



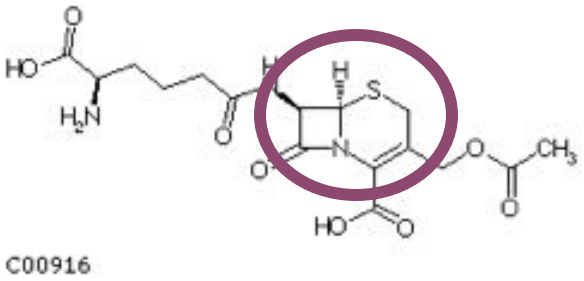
C06574

Amoxicillin

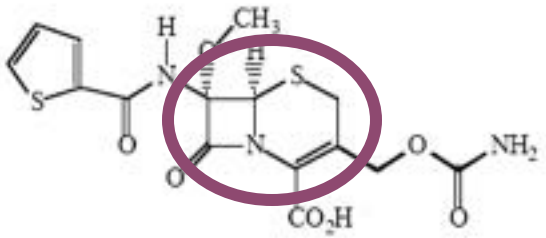


Cephalosporin's

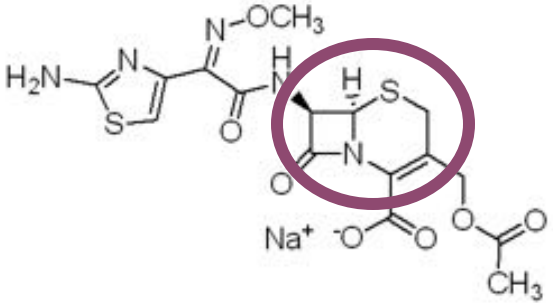
Cephalosporin C
(1. gen. Cephalosporin**)**



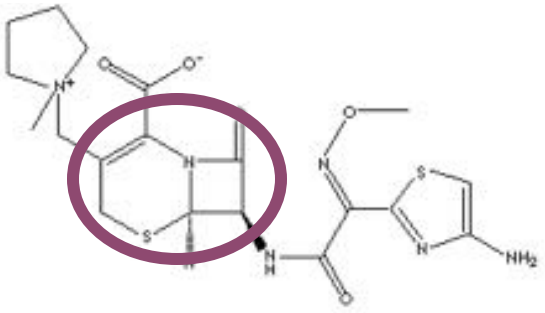
Cefoxitin (FOX)
(2. gen. cephamycin**)**



Cefotaxime (CTX)
(3. gen. Cephalosporin**)**

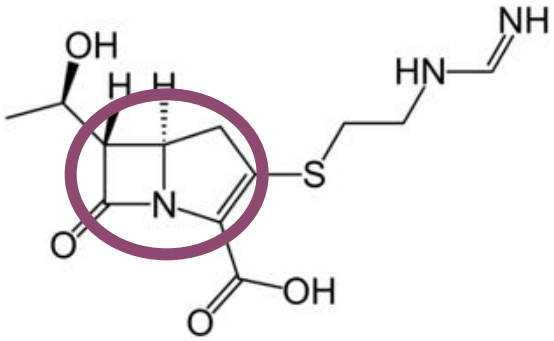


Cefepime (FEB)
(4. gen. cephalosporin**)**

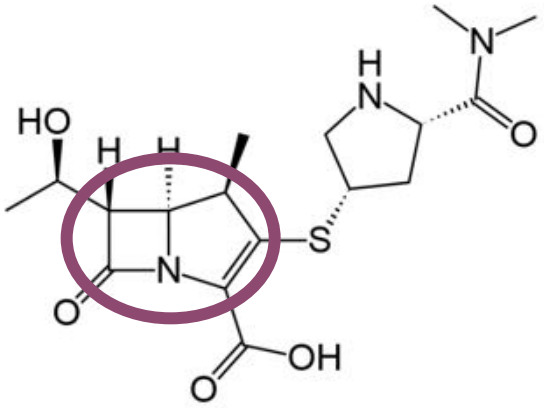


Carbapenems

Imipenem (IMI)



Meropenem (MERO)



Narrow spectrum vs. Extended spectrum Beta-lactam's

Narrow and moderate spectrum beta-lactams

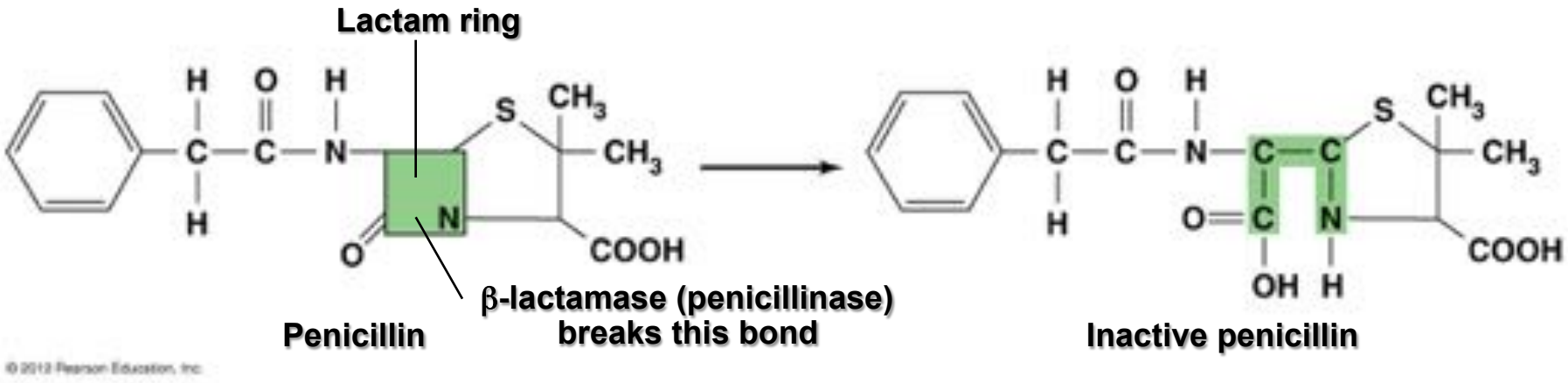
- Penicillin G and V (PEN)
- Methicillin (MET) → ***mecA* in *S. aureus***
- amoxicillin (AMOX) and ampicillin (AMP)
- Cephalotin (CEP)

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

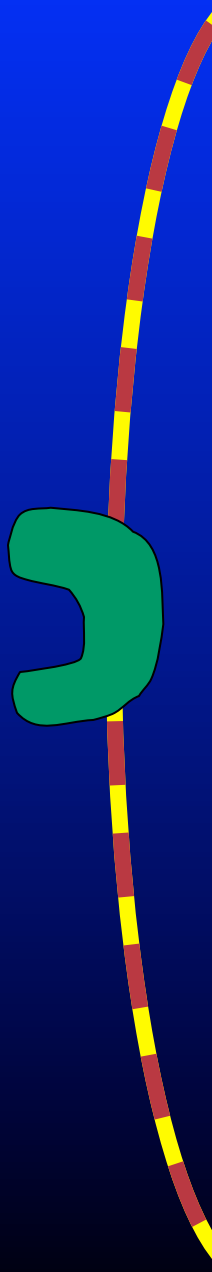
Broad and Extended spectrum beta-lactams

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

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


Lactam ring
β-lactamase (penicillinase) breaks this bond
Penicillin
Inactive penicillin
© 2012 Pearson Education, Inc.

Beta-lactam



Ampicillin

Penicillin binding protein (PBP)

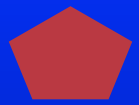
Beta-lactamase

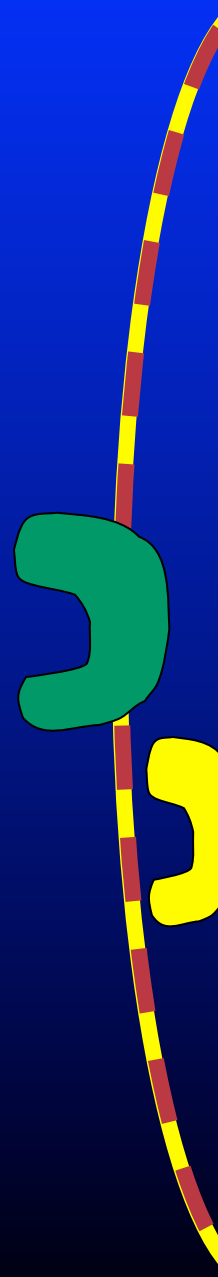
Ampicillin

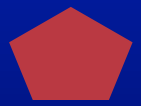
Penicillin binding protein (PBP)

Ampicillinase (TEM-1 or AmpC)

Extended-spectrum Beta-lactams





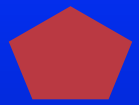


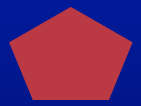
Cephalosporins (AXO)

Penicillin binding protein (PBP)

Penicillinase (TEM-1 or AmpC)

Beta-lactamase





Cephalosporins (AXO)

Penicillin binding protein (PBP)

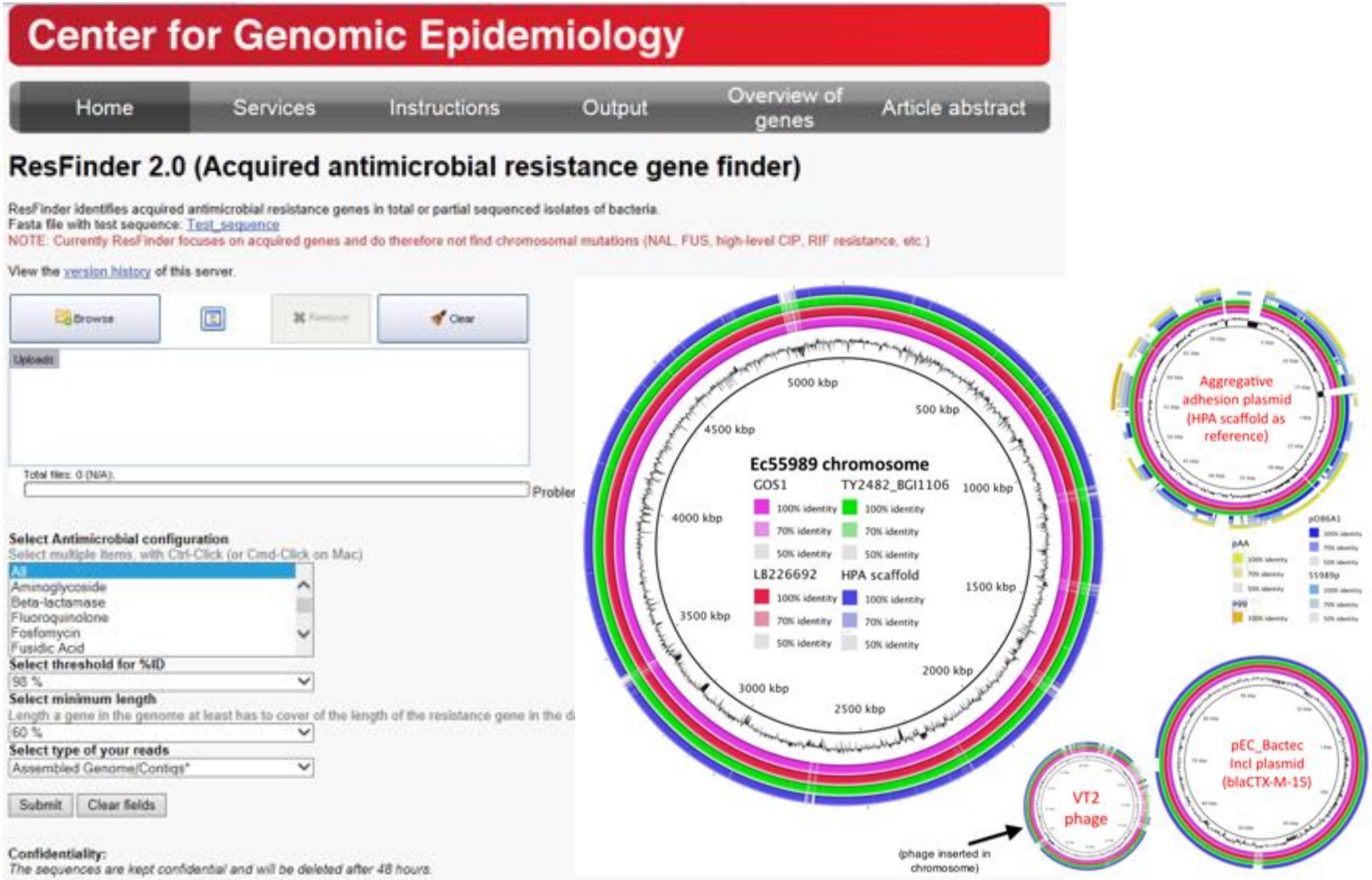
Extended-Spectrum Beta-Lactamase (SHV-12, CTX-M..)

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	

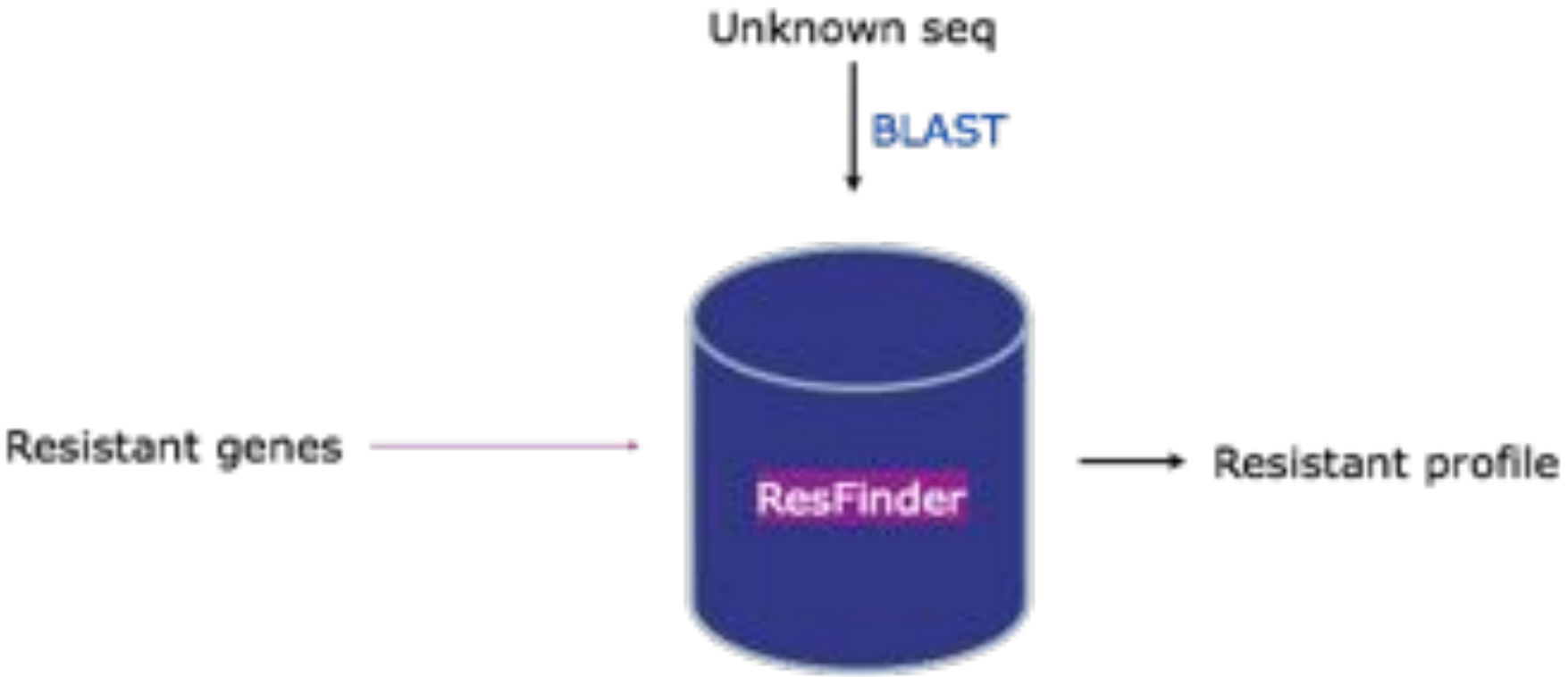
Genes in red indicate most prevalent types!

* Only some variants are cephalosporinases

Example – Resfinder



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