Antibiotic resistance

TU02

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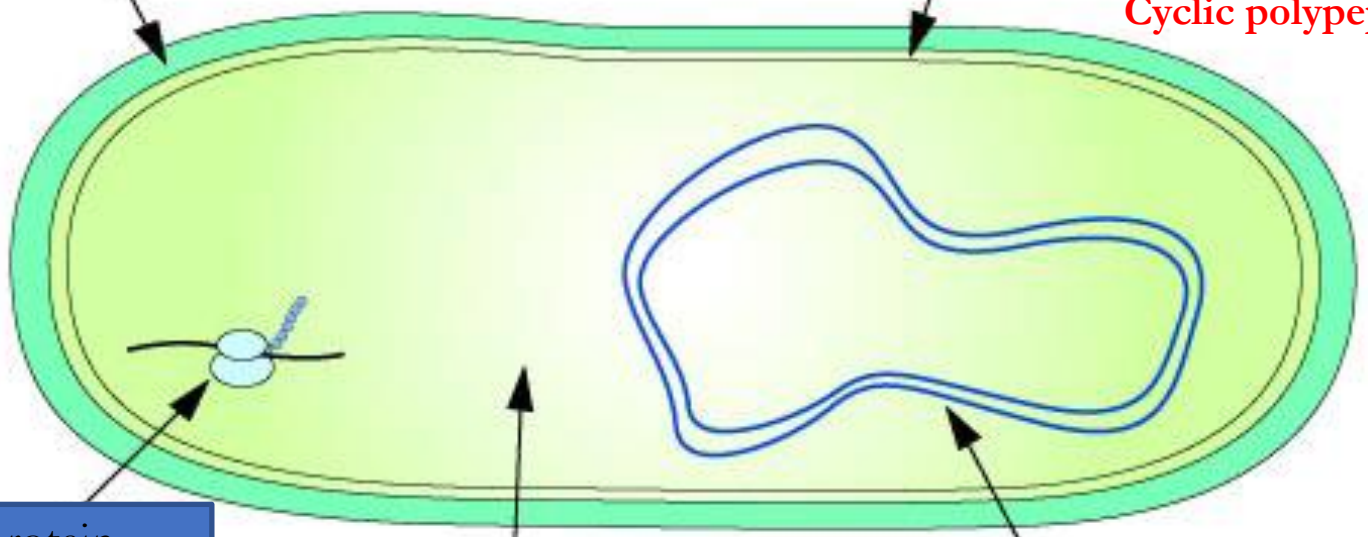
Introduction: Bacterial infections

- Around 600 bacterial species are of medical interest
- 10 species represent 75% of isolates from a hospital laboratory
  - *Staphylococcus aureus, Enterococcus faecalis, Streptococcus sp.*
  - *Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae*
  - *Pseudomonas aeruginosa*
- Around 70 antibiotics divided into 14 categories
- More than 2000 phenotypes of resistance

How the antibiotics work



1) Inhibition of cell wall synthesis

β–lactams

Glycopeptides

2) Inhibition of cytoplasmic membrane synthesis

Cyclic polypeptides

3) Inhibition of protein synthesis

Aminoglycosides

Macrolides

Other mechanisms

Rifamycines

Triméthoprime

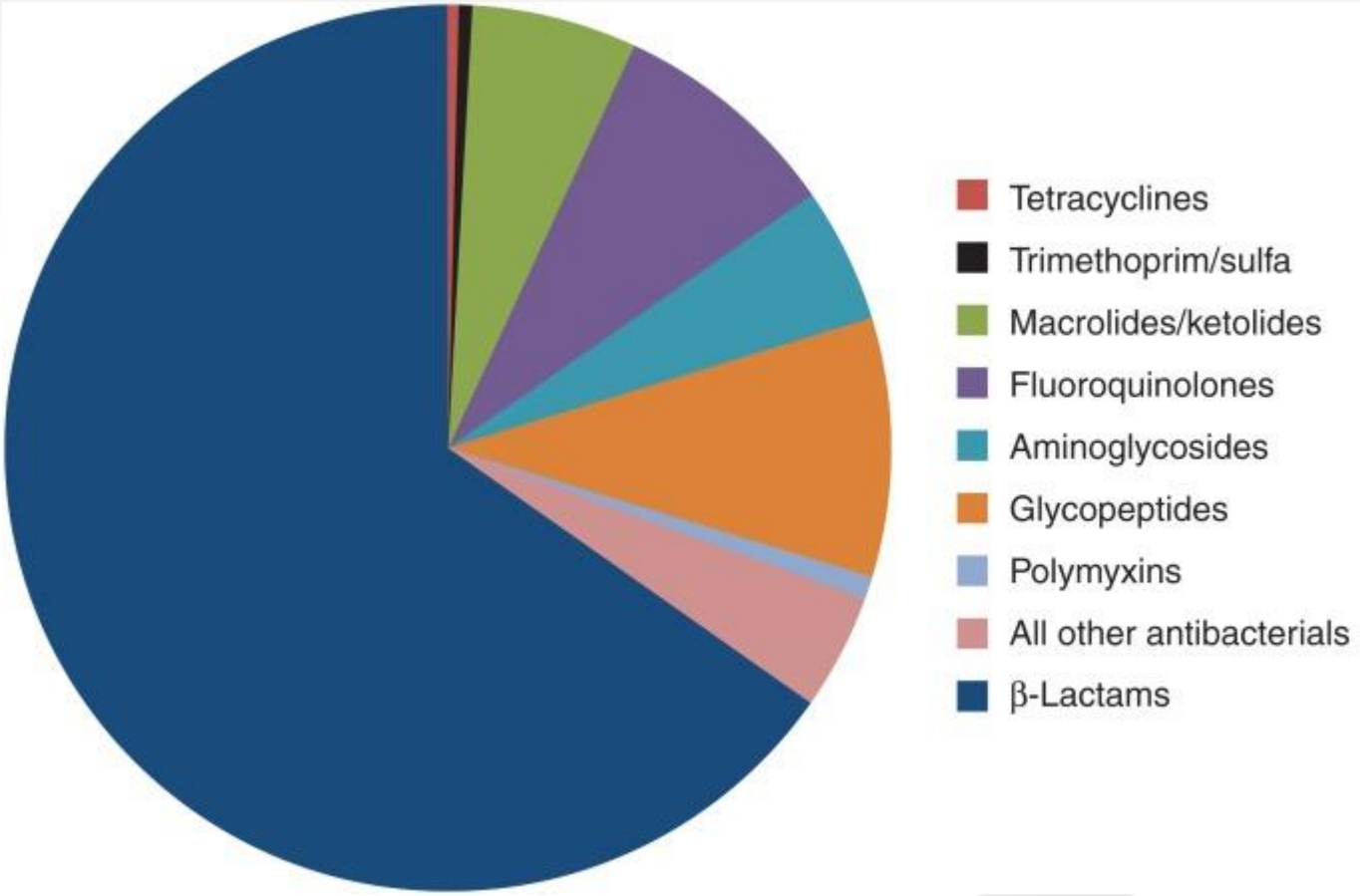
Nitrofuranes

4) Inhibition of DNA synthesis

Fluoroquinolones

Nitroimidazoles

Proportion of prescriptions in the United States for injectable antibiotics by class for years 2004–2014.



- β-lactams, 65.24%; glycopeptides, 9%; fluoroquinolones, 8%; macrolides/ketolides, 6%; aminoglycosides, 5%; polymyxins, 1%; trimethoprim/sulfamethoxazole, 0.5%; tetracyclines (excluding tigecycline), 0.4%; all other antibiotics, 4.21%. (IMS MDART Quarterly Database on file at AstraZeneca.)

Antibiotic resistant bacteria

- bacteria that are not controlled or killed by one or several antibiotics after a standard course of treatment
  - Multidrug resistant bacteria (MDR)= Resistant to at least one antibiotic in three or more antimicrobial categories
- Resistance in bacteria can be intrinsic or acquired

Intrinsic resistance

Intrinsic resistance is a naturally occurring trait characteristic of a specie, a genus….

Examples:

- **Glycopeptide resistance for Gram negative bacteria: structure**
- **Macrolides-Lincosamides-Streptogramins resistance for Gram negative bacteria : hydrophobicity**
- **Aminoglycosides resistance for anaerobic bacteria: quinone defect**
- **Penicillin resistance for genus Klebsiella: penicillinase**

Acquired resistance

- Endogenous: mutation (10-20%)
  - Antibiotic independent

- Exogenous: acquisition of foreign DNA (80-90%)
  - Transformation

  - Conjugation with mobile genetic elements

  - Conjugative plasmids, mobilizable
  - Conjugative transposons, mobilizable
  - Integrons

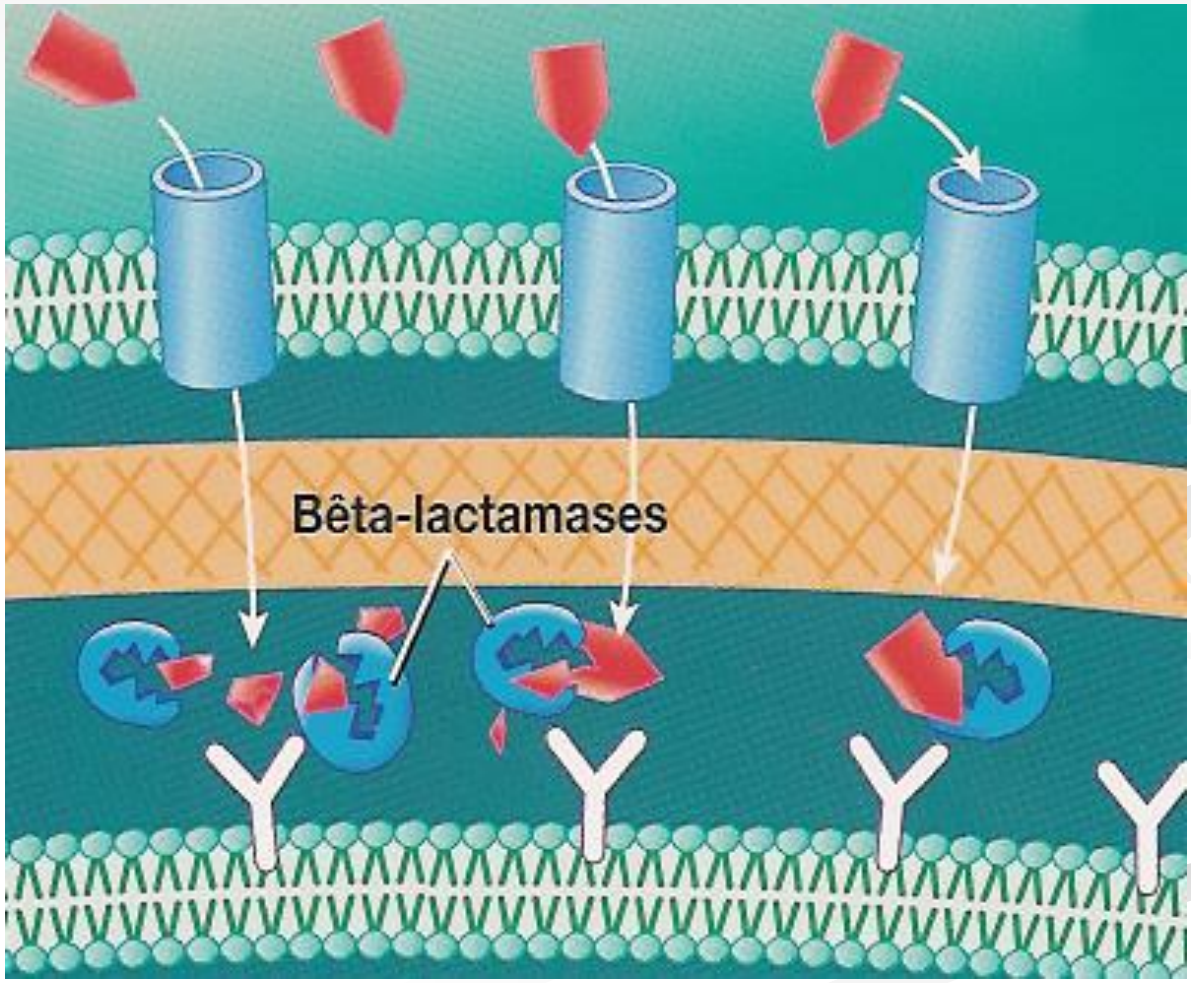
  - Transduction with bacteriophage

I. Mechanisms of resistance to antibiotics

- Production of inactivating enzymes
- Changing the target
- Membrane impermeability
- Efflux (excretion) of the antibiotic by enzymatic systems

Lack of entry of the antibiotic to reach its target

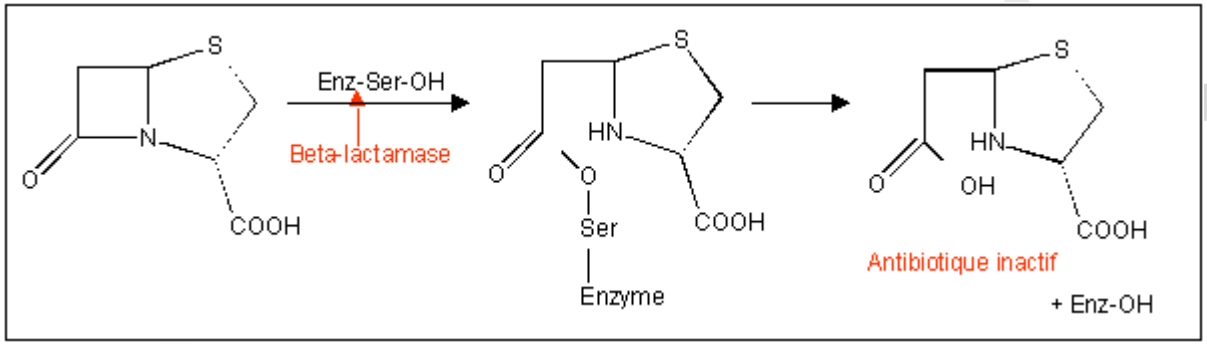
1) Production of inactivating enzymes



A) Enzymes targeting β-Lactam antibiotics

- **β -Lactamases**
  - gene present on the bacterial chromosome or may be acquired via plasmid transfer
  - β-lactamase gene expression may be induced by exposure to β-lactams
  - can be found either extracellularly or within the periplasmic space of bacteria

- hydrolysis of the β-lactam ring: → inactivation



Different substrats for the β-lactamases

Penicillins

Péni.G, V

Peni M: Oxacillin

Peni A: Amoxicillin

CarboxyP: Ticarcillin

UreidoP: Piperacillin

Aztreonam (monobactam)

Carbapenems: Imipenem..;

Cephalosporins

C1G:

Cephalothin

C2G:

Cefuroxim

Cefamandole

Cefoxitin (céphamycine)

Cefotetan (céphamycin)

C3G:

Cefotaxime

Ceftazidime

Ceftriaxone

C4G:

Cefepime

Cefpirome

C5G ?:

Ceftaroline

Ceftobiprole

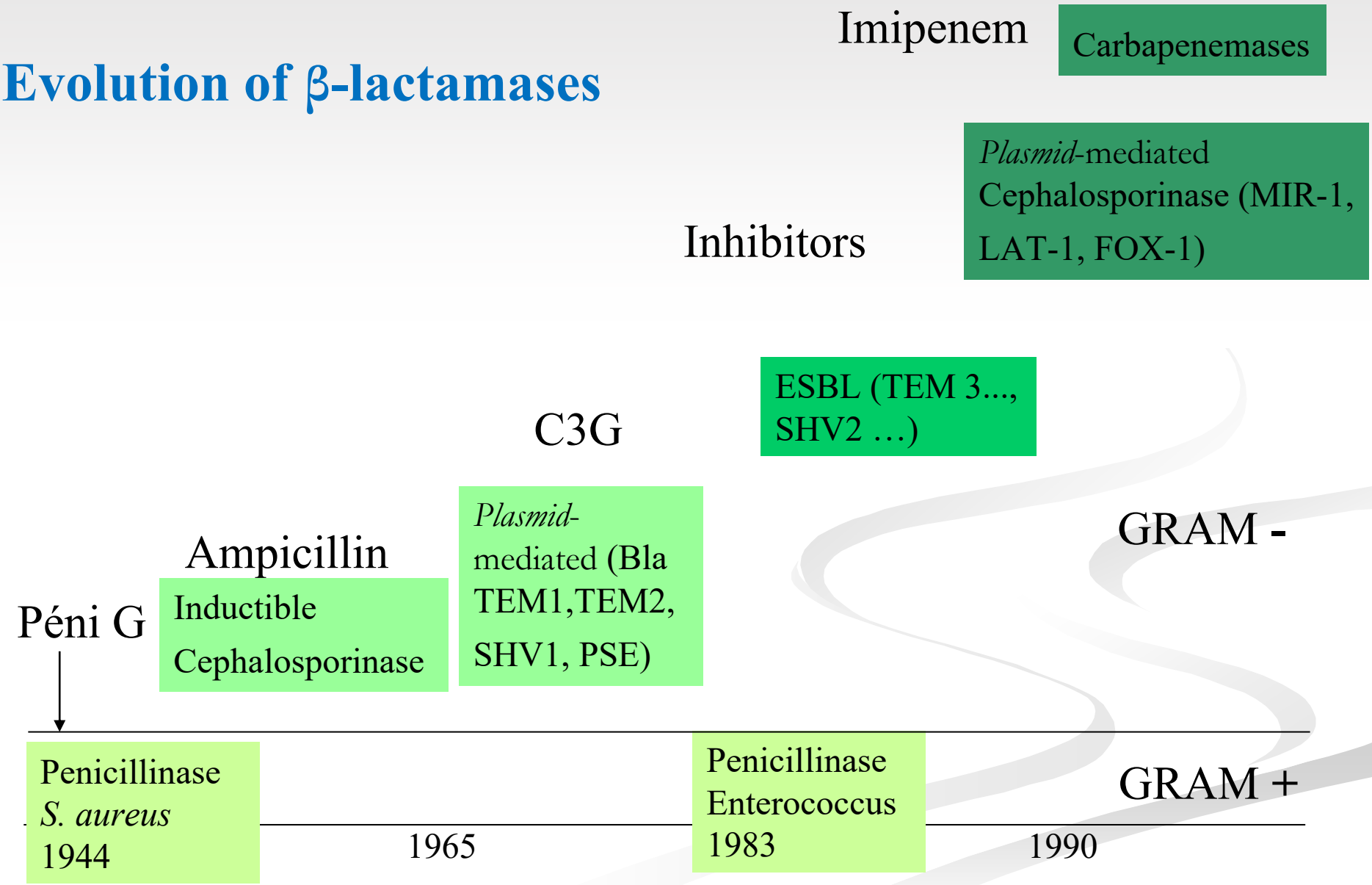
Classification of the β-lactamases

- Ambler : Molecular (based on nucleotide sequence)

Type	Ambler Molecular Class	Characteristics	Examples of Enzymes
Narrow-spectrum β-lactamases[12,18,19]	A	Hydrolyze penicillin; produced primarily by *Enterobacteriaceae*	Staphylococcal penicillinase, TEM-1, TEM-2, SHV-1
Extended-spectrum β-lactamases[20]	A	Hydrolyze narrow and extended-spectrum β-lactam antibiotics	SHV-2, CTX-M-15, PER-1, VEB-1
Serine carbapenemases[20]	A	Hydrolyze carbapenems	KPC-1, IMI-1, SME-1
Metallo-β-lactamases[21,22]	B	Hydrolyze carbapenems	VIM-1, IMP-1, NDM-1
Cephalosporinases[10,23,24]	C	Hydrolyze cephamycins and some oxyimino β-lactams; inducible; chromosomally mediated	AmpC, P99, ACT-1, CMY-2, FOX-1, MIR-1
OXA-type enzymes[25-27]	D	Hydrolyze oxacillin, oxyimino β-lactams, and carbapenems; produced by *Pseudomonas aeruginosa* and *Acinetobacter baumannii*	OXA enzymes

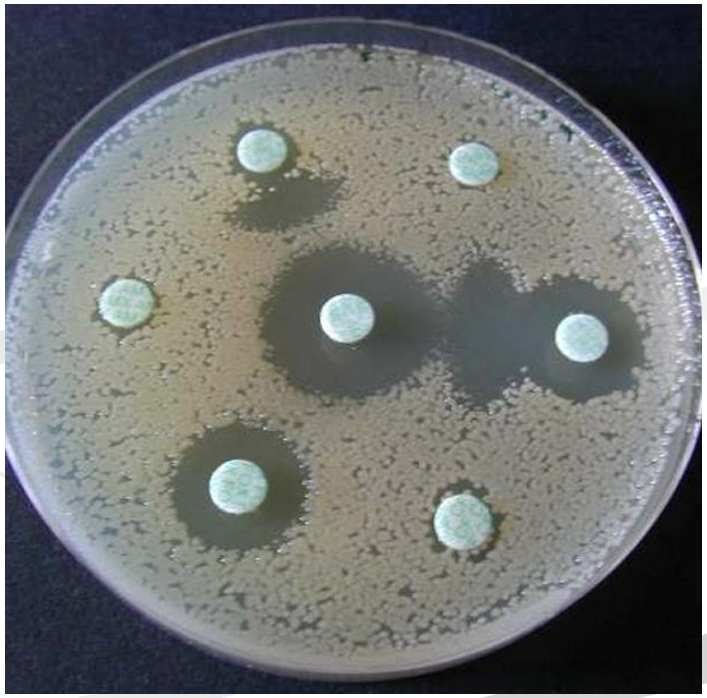
- > 900
- Metallo β-lactamases are structurally similar to RNase Z
- Serine beta-lactamases have evolved from DD-transpeptidases (penicillin-binding proteins)

Evolution of β-lactamases

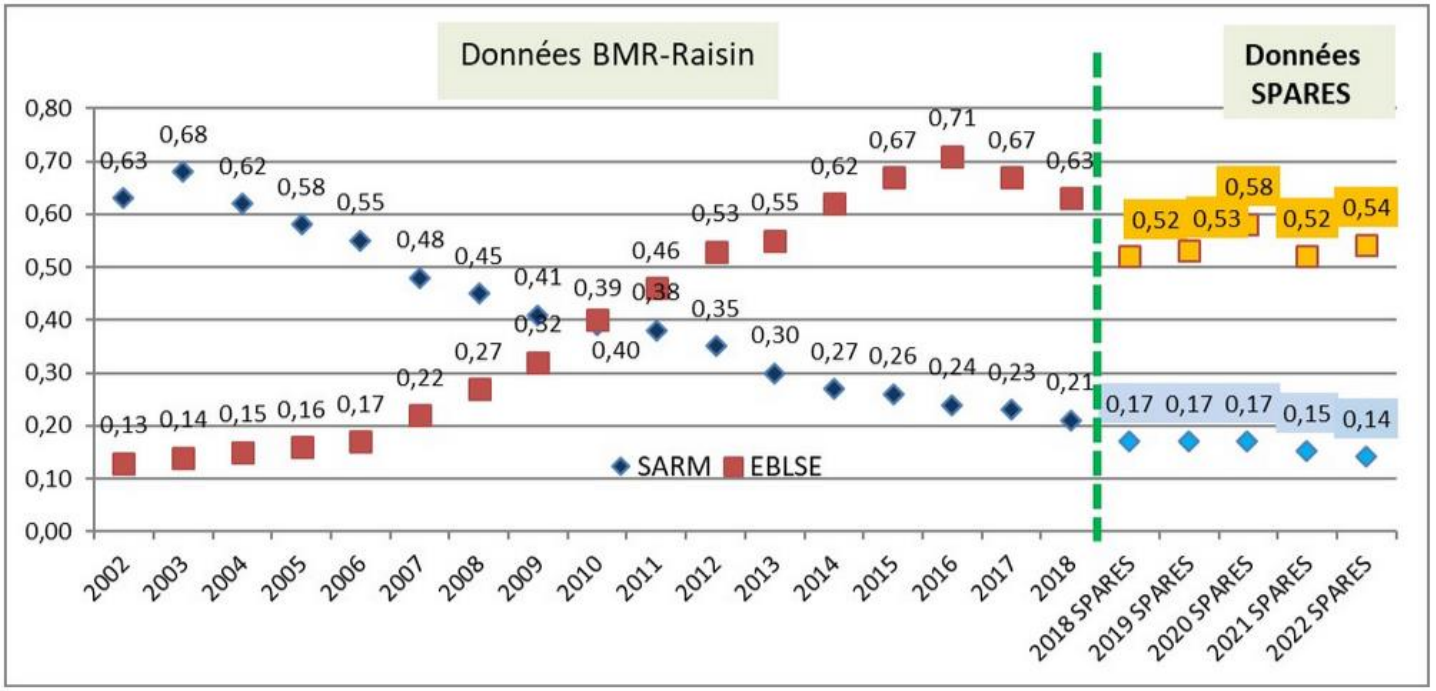


Focus on the Extended-spectrum β-lactamases (ESBL)

- Some enzymes have a specificity directed towards the cephalosporines of third generation.
- Active on all β-lactams except cephamycins and carbapenems.
- Synergy test (double disc with B-lactamase inhibitors) = « champagne cork ».
- Plasmid origin: transferable (diffusion and evolution = 200 ESBL).



- ESBL-producing bacteria cost the highest for community-onset and hospital-onset infections.
- Patients with ESBL-producing bacteremia = higher mortality rate
- Increase in the worldwide community prevalence



New carbapenemase: NDM-1

- New Delhi metallo-β-lactamase 1.
- Confers resistance to all β-lactams.
- Isolated first from Klebsiella and from other Enterobacteriaceae.
- Carried by a plasmid with other resistance genes (=MDR): strains are resistant to all antibiotics except polymyxins (cyclic peptide antibiotics).

Inhibitors of β-lactamases

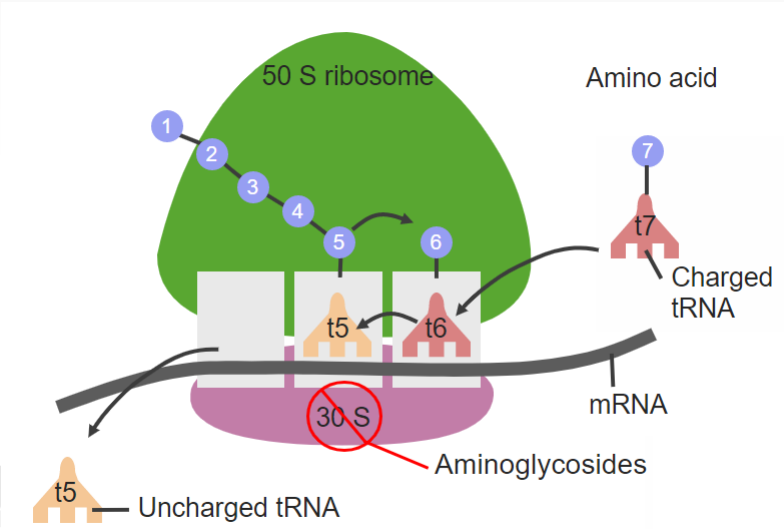
Suicide inhibitor. Covalently bonded to a serine residue in the active site of the β-lactamase.

Clavulanic acid + Amoxicillin
+ Ticarcillin (hospital)

Tazobactam + Piperacillin = Tazocin (hospital)

B) Enzymes targeting aminoglycosides

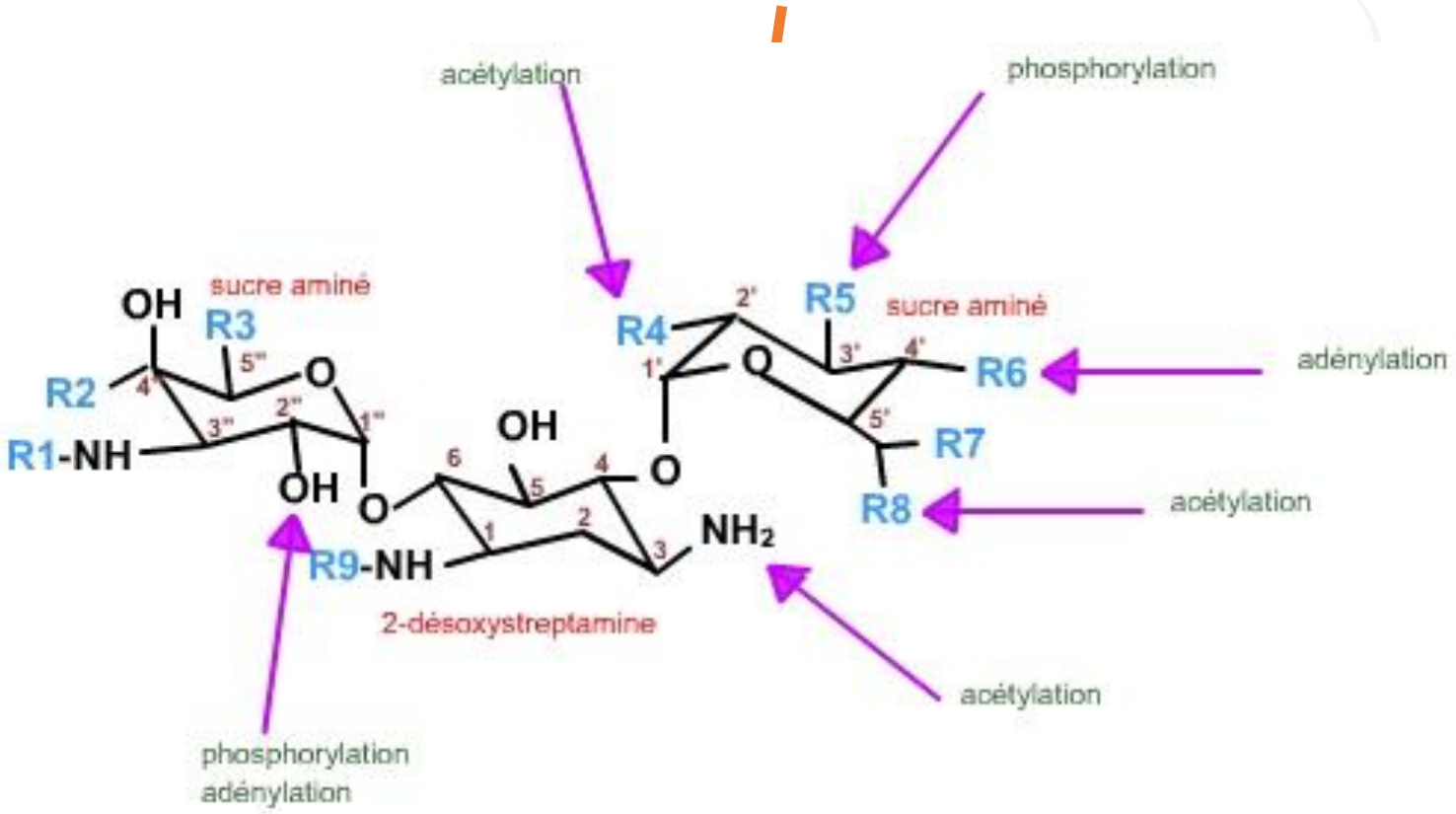
- Aminoglycoside: gentamicin, amikacin, tobramycin, neomycin…
- Broad-spectrum antibacterial activity



- **Target:** Aminoglycosides inhibit protein synthesis by binding with high affinity to the A-site on the 16S ribosomal RNA of the 30S ribosome subunit

Enzymes targeting aminoglycosides

- Aminoglycosides have amino or hydroxyl groups which can be the target of three classes of enzymes:
  - Phosphotransferase (APH),
  - Adenylyl-transferase (AAD or ANT)
  - and Acetyltransferase (AAC)

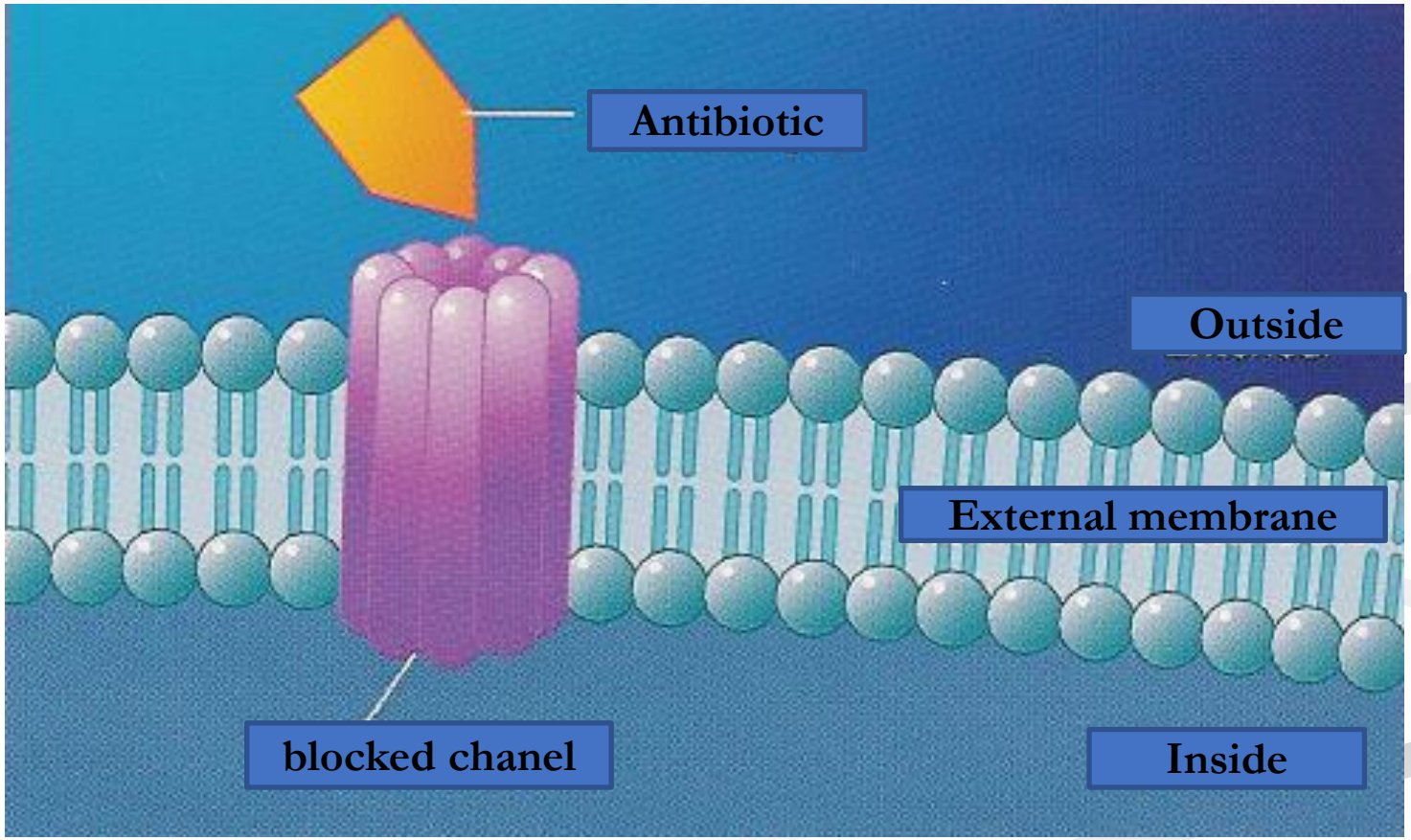


Examples of inactivating enzymes of aminoglycosides

Antibiotic/ enzyme	Kanamycin	Tobramycin	Amikacin	Gentamicin	Netilmicin
APH(3')-I, II	R	S	S	S	S
APH(3')-VI	R	S	R	S	S
AAC(2')-I	S	I/R	S	I/R	I/R
AAC(3)-II	S	R	S	R	R
AAC(6')-I	R	R	R	S	R
AAC(6')-II	R	R	S	R	R
ANT(2")	R	R	S	R	S
ANT(4')-II	I/R	R	I/R	S	S

R=Resistant; I= intermediate; S=sensible

2) Impermeability



- **Result in the change of the porin-mediated outer membrane (OM) permeability (gram negative bacteria)**

Porins provide a path through the OM to small hydrophilic antibiotics (β-lactams, chloramphenicol, fluoroquinolones…)

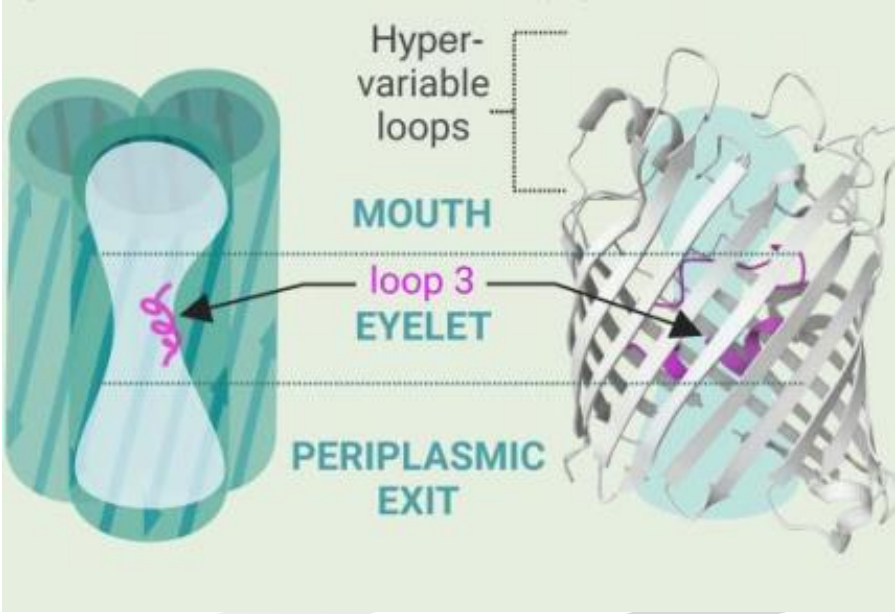
3 strategies of resistance:

- Regulation and balance of porins
  - Different system of regulation of gene expression (Two component system, transcriptional regulator, extracytoplasmic sigma factor)
- Substitution of a narrower porin
  - reduced permeation rate = A hot spot for single or multiple mutations
- LPS alteration can induce improper assembly or misfolding during insertion, leading to porin degradation

Ex: Key mutations altering channel diameter (Loop3)

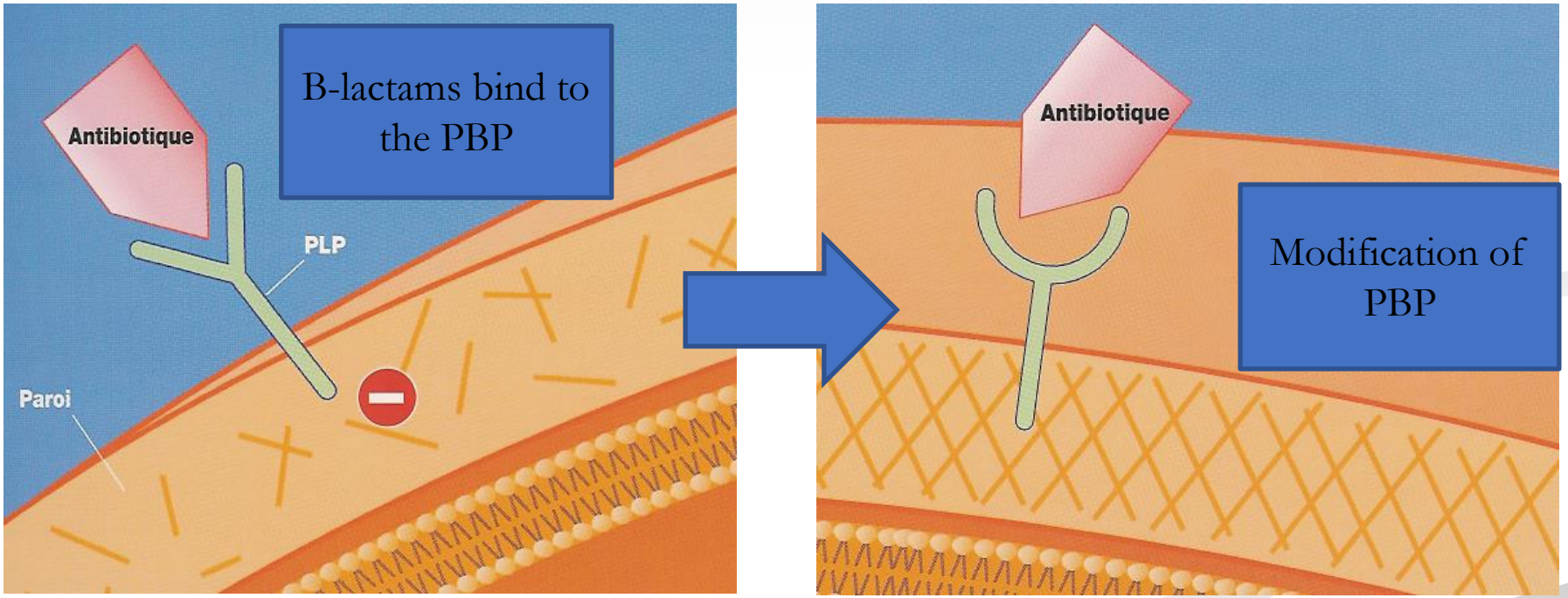
- Three regions constitute the porin channel: the mouth, the eyelet and the periplasmic exit

- The first mutation, a G119D substitution, was described in *E. coli* OmpF obtained by selecting for resistance to colicin



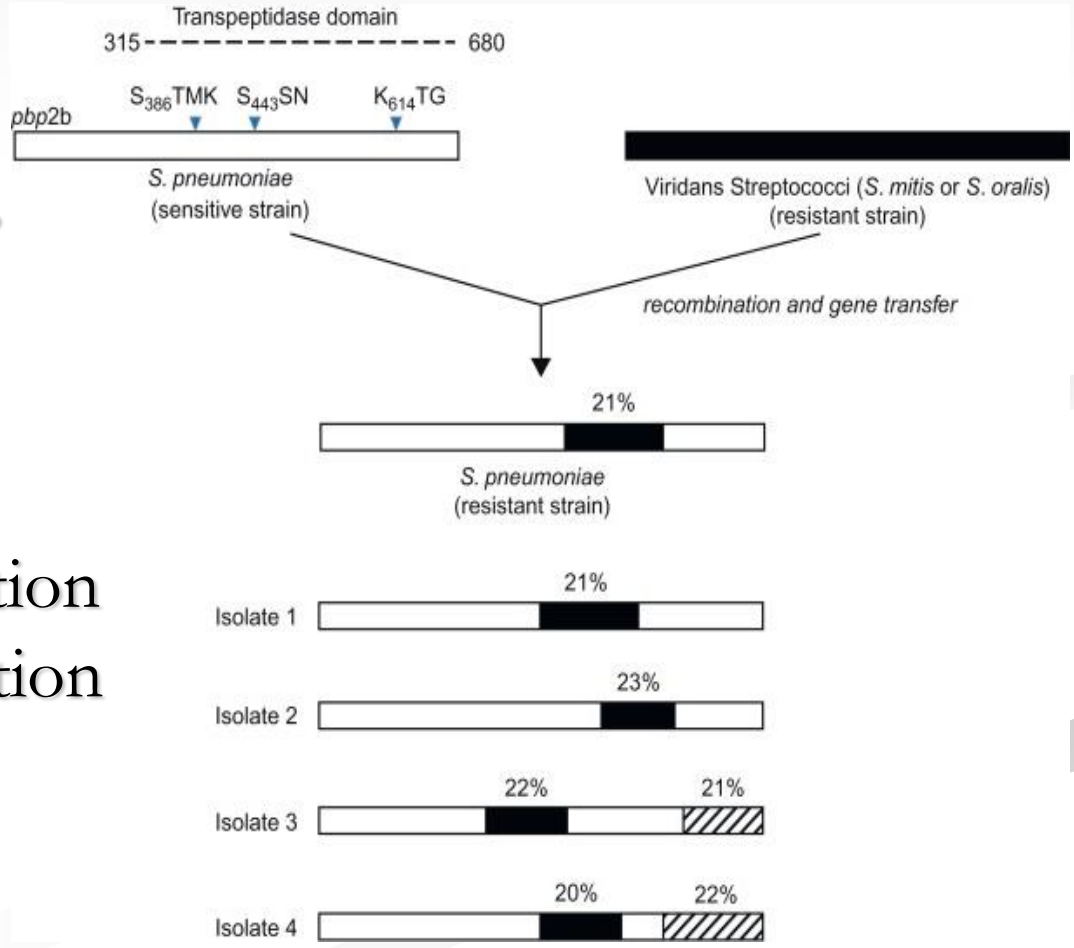
- *J Antimicrob Chemother* 2024; **79**: 2460–2470

3) Modification of the target



a) Resistance to penicillin

1) Penicillin-sensitive *Streptococcus pneumoniae* can incorporate regions of altered *pbp* genes from commensal streptococci (*S. mitis, S. oralis*) by transformation and homologous recombination

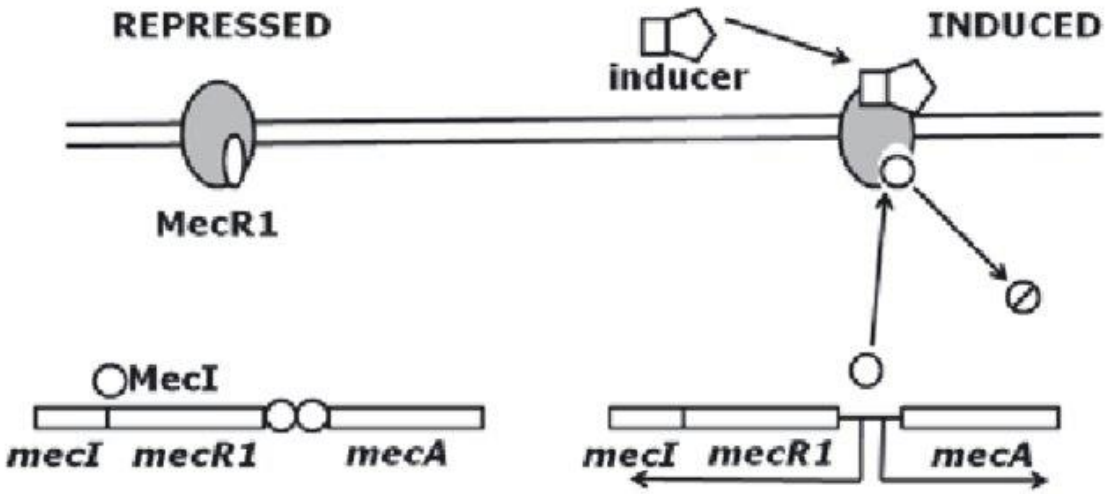


2) Methicillin resistant *Staphylococcus aureus*

Resistance due to acquisition of the mecA gene, which expresses a unique penicillin-binding protein (PBP2a) with low affinity for methicillin and other beta-lactams

Expression of PBP2a is controlled by a regulation system, including a repressor MecI and a receptor MecR1. MecI represses transcription of mecA

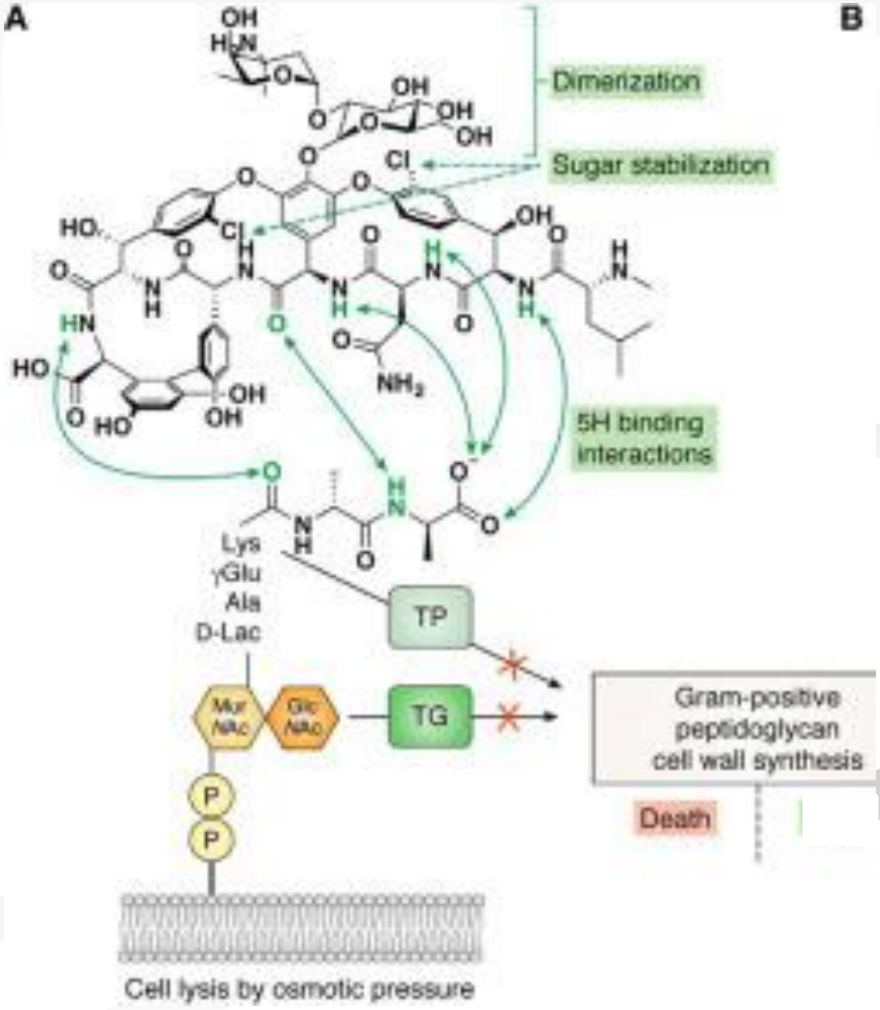
Upon binding of β-lactam to sensor domain of MecRI, intracellular peptidase domain cleaves MecI repressor, which triggers mecA and mecI transcription



b) Resistance to glycopeptides

- Glycopeptides are peptides that contain carbohydrate moieties (glycans) covalently attached to the side chains of the amino acid residues that constitute the peptide
- They act by binding to the D-Ala-D-Ala dipeptide terminus of the peptidoglycan precursors:

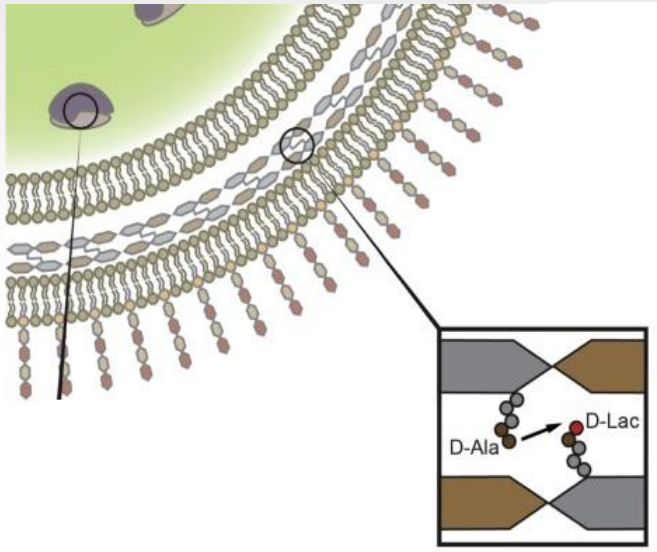
the substrate is sequester from transpeptidation and transglycosylation

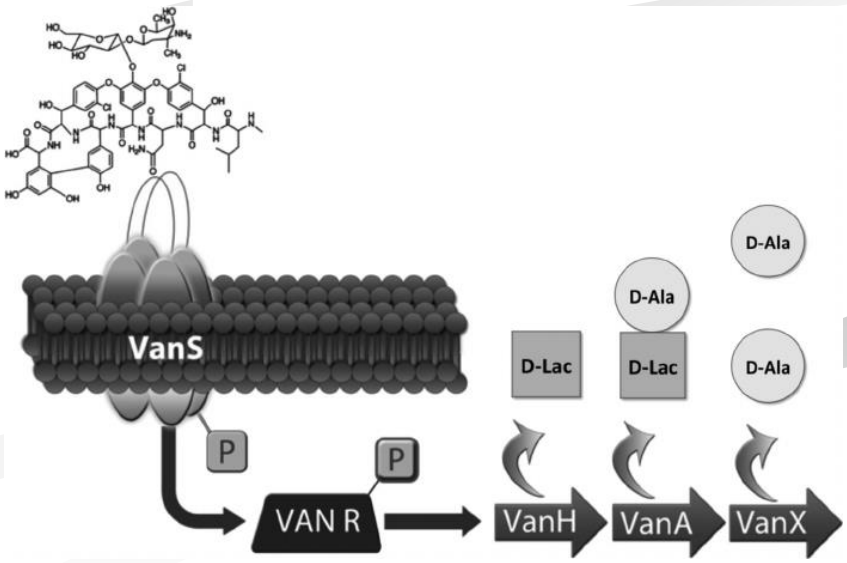


Example : vancomycin

Reduction of antibiotic affinity through cell wall modification

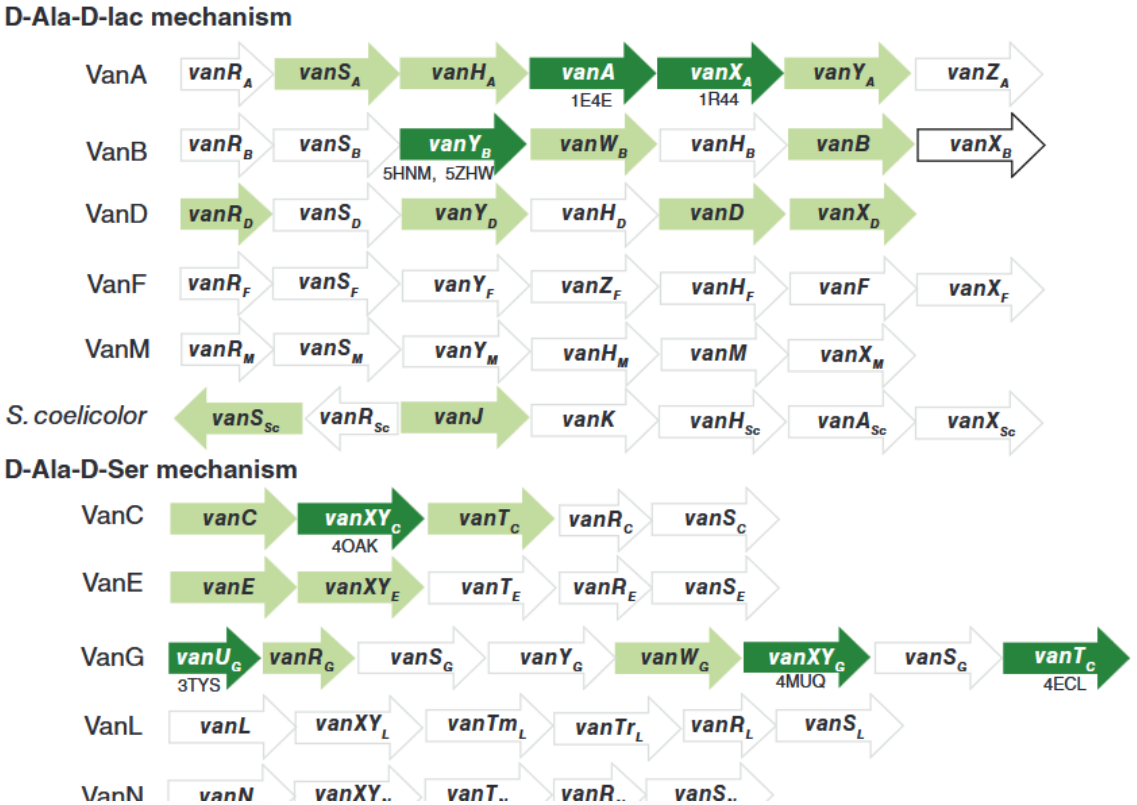
- Change in peptidoglycan precursor D-alanine-D-alanine to precursors like D-alanine-D-lactate or D-alanine-D-serine
- Results in a 1,000-fold decrease in binding constant between vancomycin and peptidoglycan
- Due to the acquisition of an operon (van): a two component system, a dehydrogenase (reduces pyruvate to D-Lactate), a ligase (synthesizes D-alanine-D-lactate) and a dipeptidase. (hydrolyses D-alanine-D-alanine precursors).
- rapid emergence and spread of vancomycin resistance amongst Gram-positive bacteria (enterococcus spp)





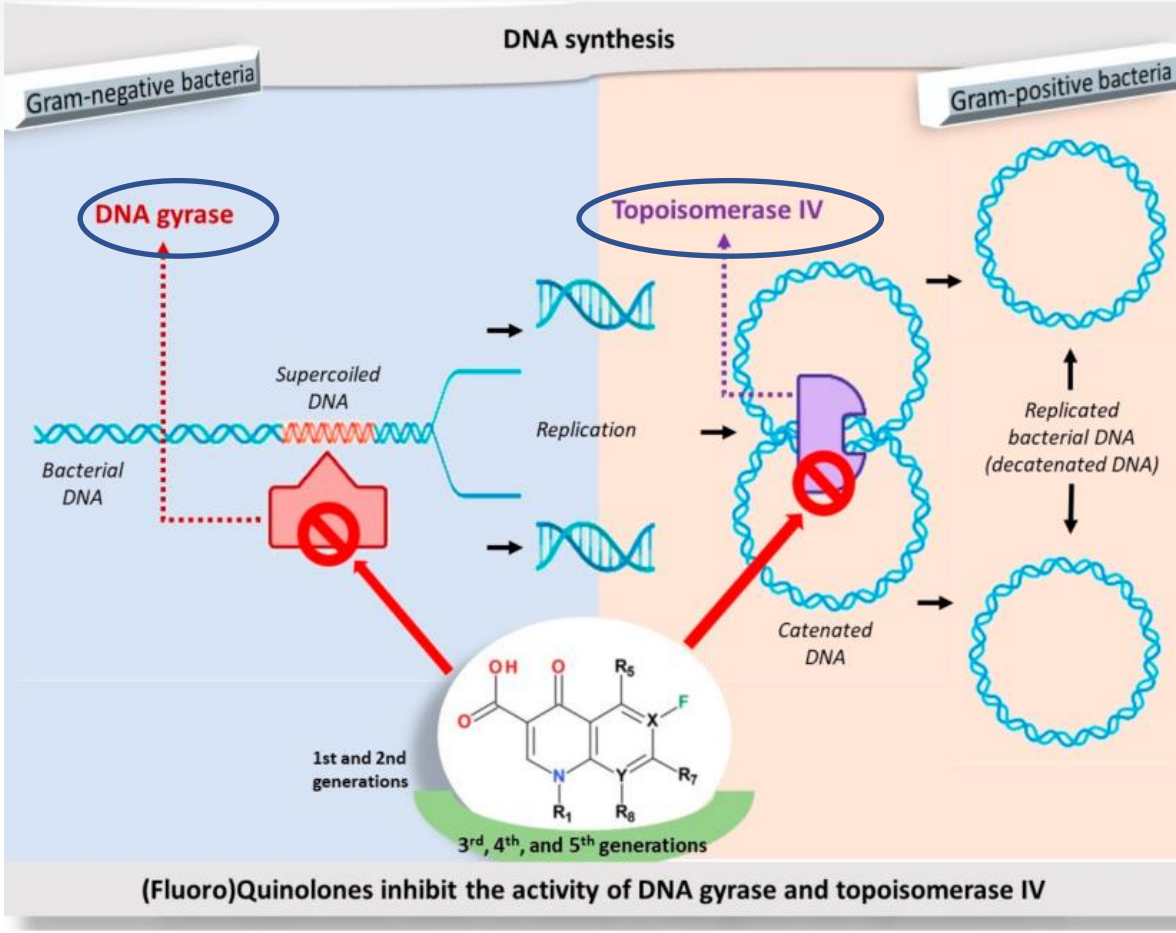
Several operon van

- VanA to N
- Carried by conjugative plasmid or transposon : ex tn1549 (vanB)



c) Resistance to fluoroquinolones

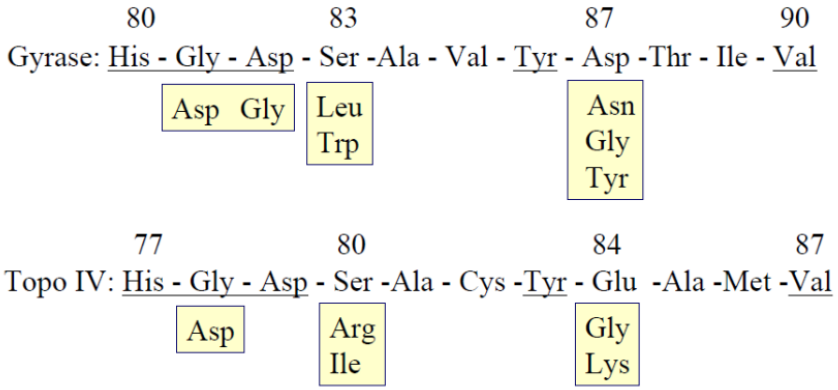
- Quinolone: Isolated during the synthesis of chloroquine (antimalarial agent) with bactericidal action
- The second generation, "fluoroquinolones" has a fluorine atom in the sixth position of the quinolinic nucleus = improved biological activity.
- Fluoroquinolones inhibit two enzymes used for DNA synthesis : DNA gyrase and topoisomerase IV



Rusu, A et al. Pharmaceutics 2021, 13, 1289.

Resistance is due to chromosomal mutations

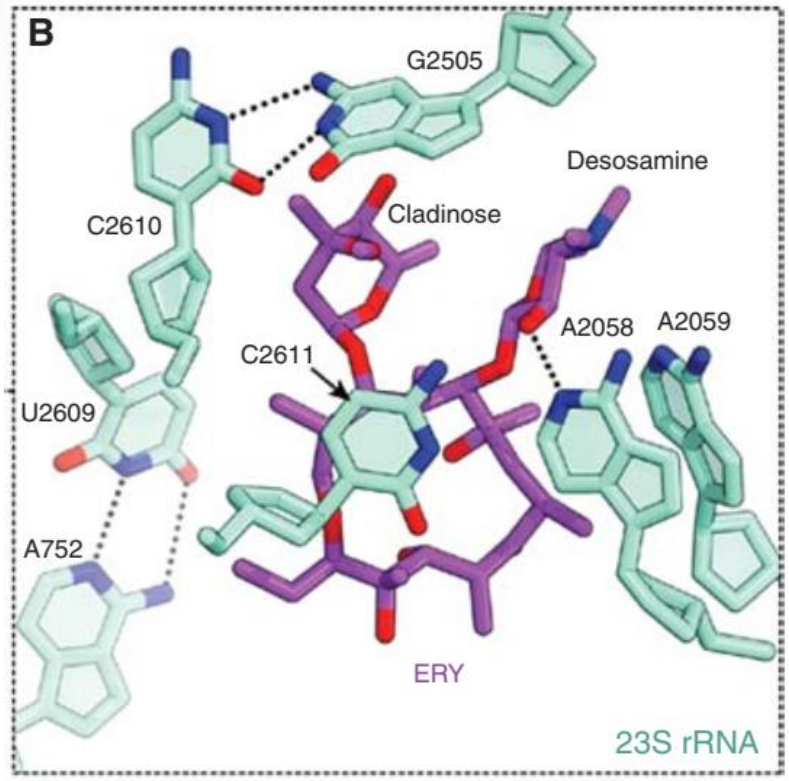
- Due to errors in the genes encoding the GyrA subunits of DNA gyrase and ParC (or ParE) of topoisomerase IV resulting in decreased target affinity
- Mutations localized to the amino terminal domains of GyrA or ParC. In proximity to the active site which are covalently linked to DNA
- Domain named the *quinolone resistance determining region* (QRDR) of GyrA and ParC.



d) Enzymes targeting macrolide target

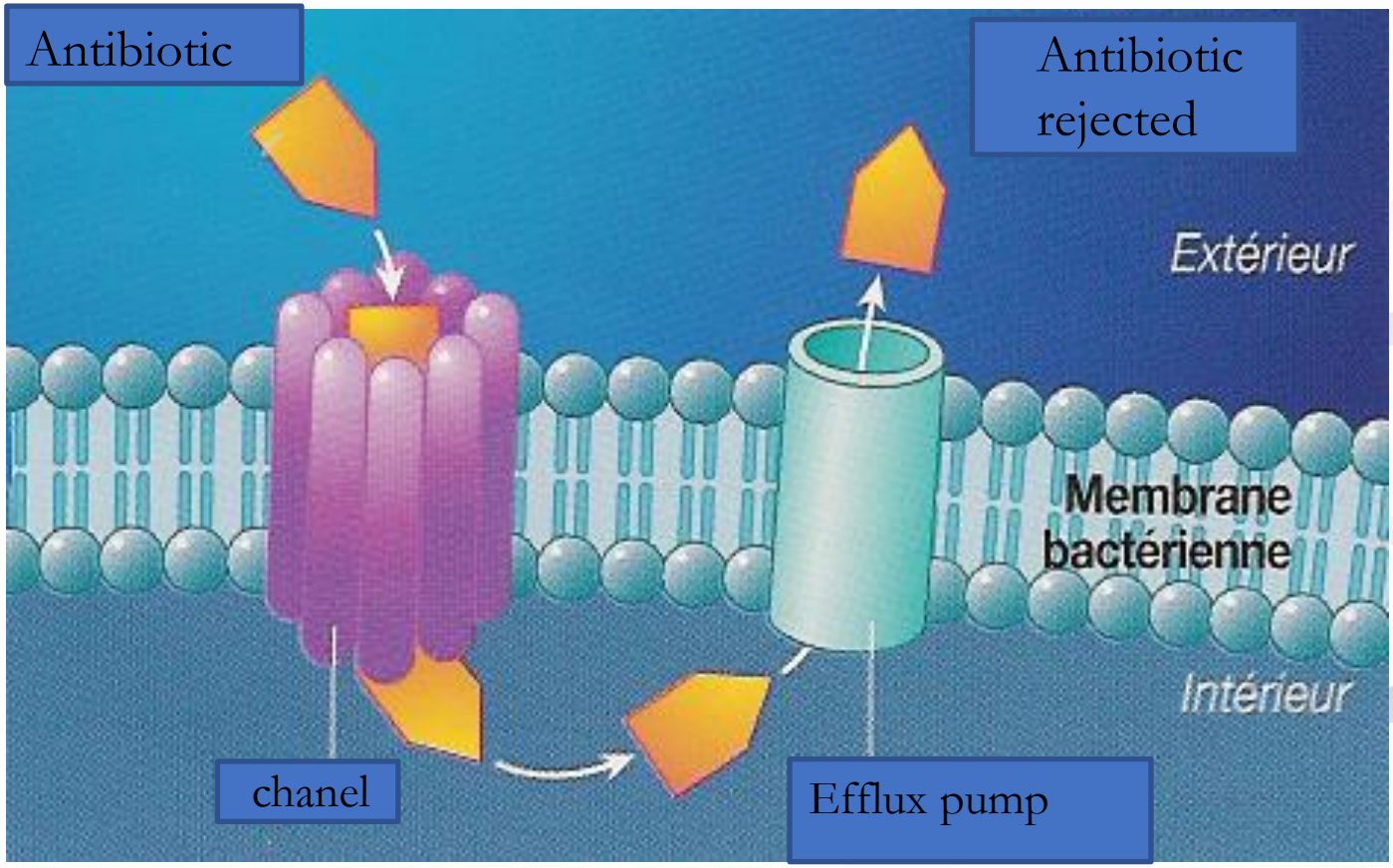
- Macrolides/ketolides are sensed by the ribosome and selectively inhibit protein synthesis by binding to the 23S ribomomal RNA.

- Erm methyltransferases add one or two methyl groups to the N-6 exocyclic amino group of A2058 of the 23S ribosomal RNA
- leads to co-resistance to macrolides, lincosamides and streptogramins type B (MLSB phenotype).



- Disruption the key hydrogen bond between A2058 and the desosamine sugar at C5
- Cold Spring Harb Perspect Med 2016;6

4) Presence or increased number of efflux-pump



Definition of the bacterial efflux pump

- An efflux pump is an active transporter in cells that moves out molecules, including antimicrobial agents, metabolites and quorum sensing signal molecules.
- Found in gram positive or gram negative bacteria
- All bacterial genomes studied contain several different efflux pumps indicating their ancestral origins.

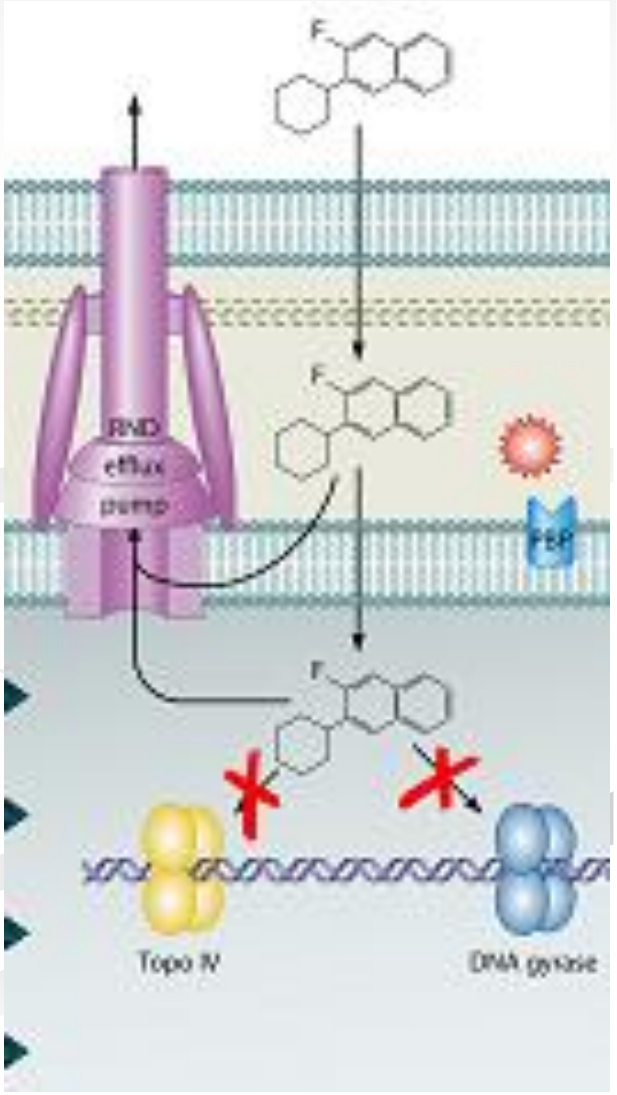
- Five families, including:
  - major facilitator superfamily (MFS),
  - small multidrug resistance (SMR),
  - ATP-binding cassette (ABC transporter),
  - resistance nodulation division efflux pumps (RND HAE),
  - the multidrug and toxic compound extrusion (MATE) transporters

- Efflux systems described from a number of clinically important bacteria:
  - *Campylobacter jejuni* (CmeABC),
  - *E. coli* (AcrAB-TolC, AcrEF-TolC, EmrB, EmrD),
  - *Pseudomonas aeruginosa* (MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY-OprM),
  - *Streptococcus pneumoniae* (PmrA),
  - *Salmonella typhimurium* (AcrB11),
  - *Staphylococcus aureus* (NorA).

Example: Efflux pump type RND

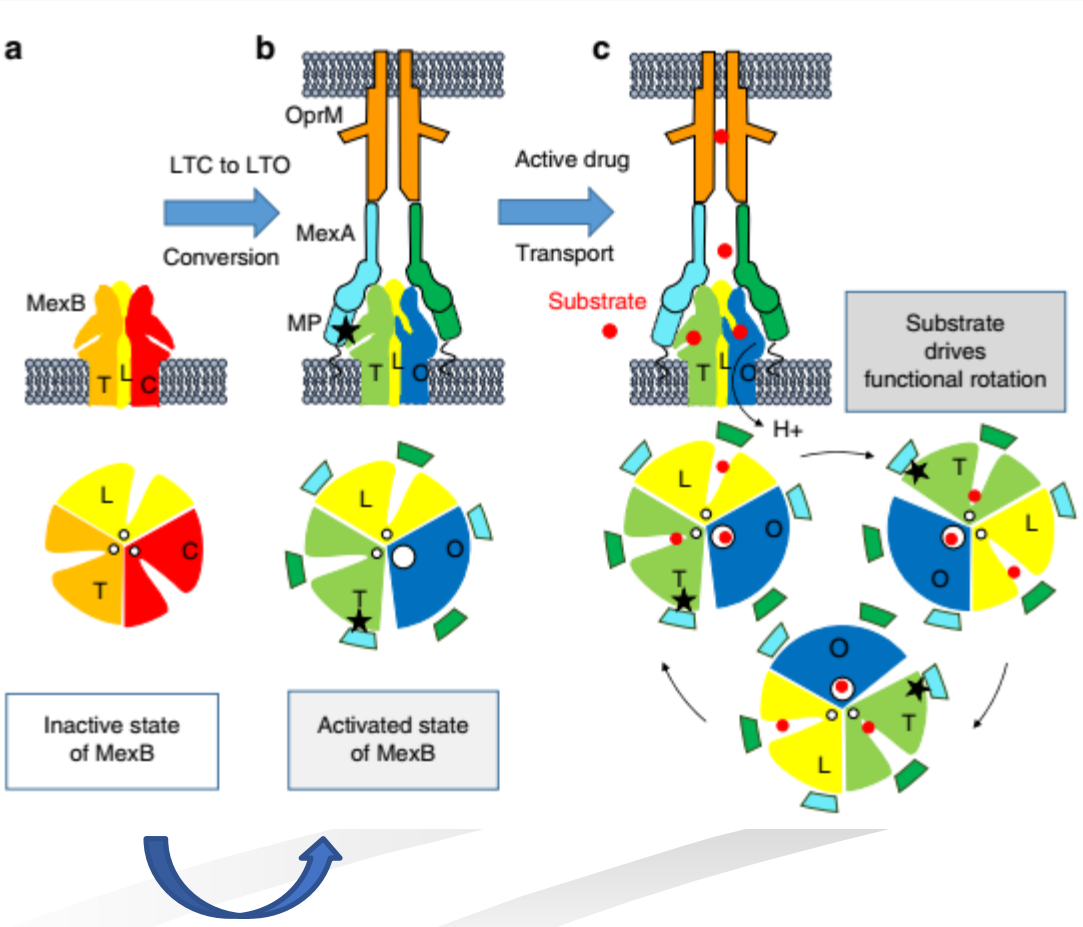
(Resistance Nodulation cell Division)

- 3 components :
  - transport protein in the cytoplasmic membrane
  - protein in the periplasm forming a channel connecting the two membranes (MFP)
  - protein in the outer membrane like porin expelling the substrate (OMF)
  - Operate as a secondary proton/drug



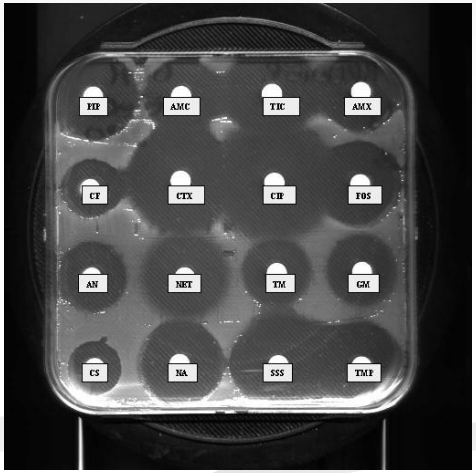
Ex: MexAB-OprM (***P. aeruginosa***) confers resistance to a broad spectrum of antibiotics.

- MexB transporter works in conjunction with OprM (OMF component), and MexA (MFP component).
- MexB as well as the E coli homologous AcrB are asymmetric homotrimers for which the three monomer conformations representing consecutive states were designated Loose(or Access), Tight (or Binding), and Open (or Extrusion)

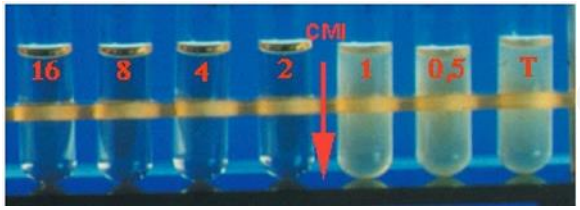


II. Detection of a mechanism of resistance

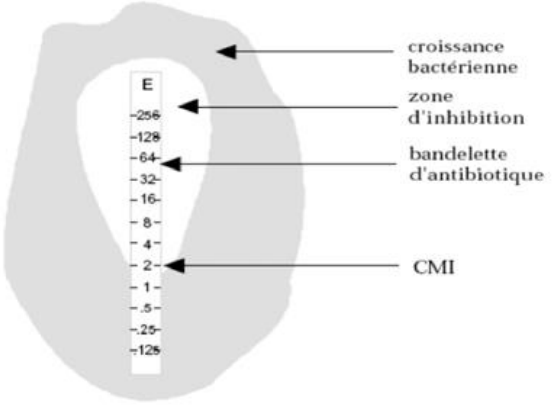
- **By disk diffusion methodology**



- Broth micro-dilution (BMD)



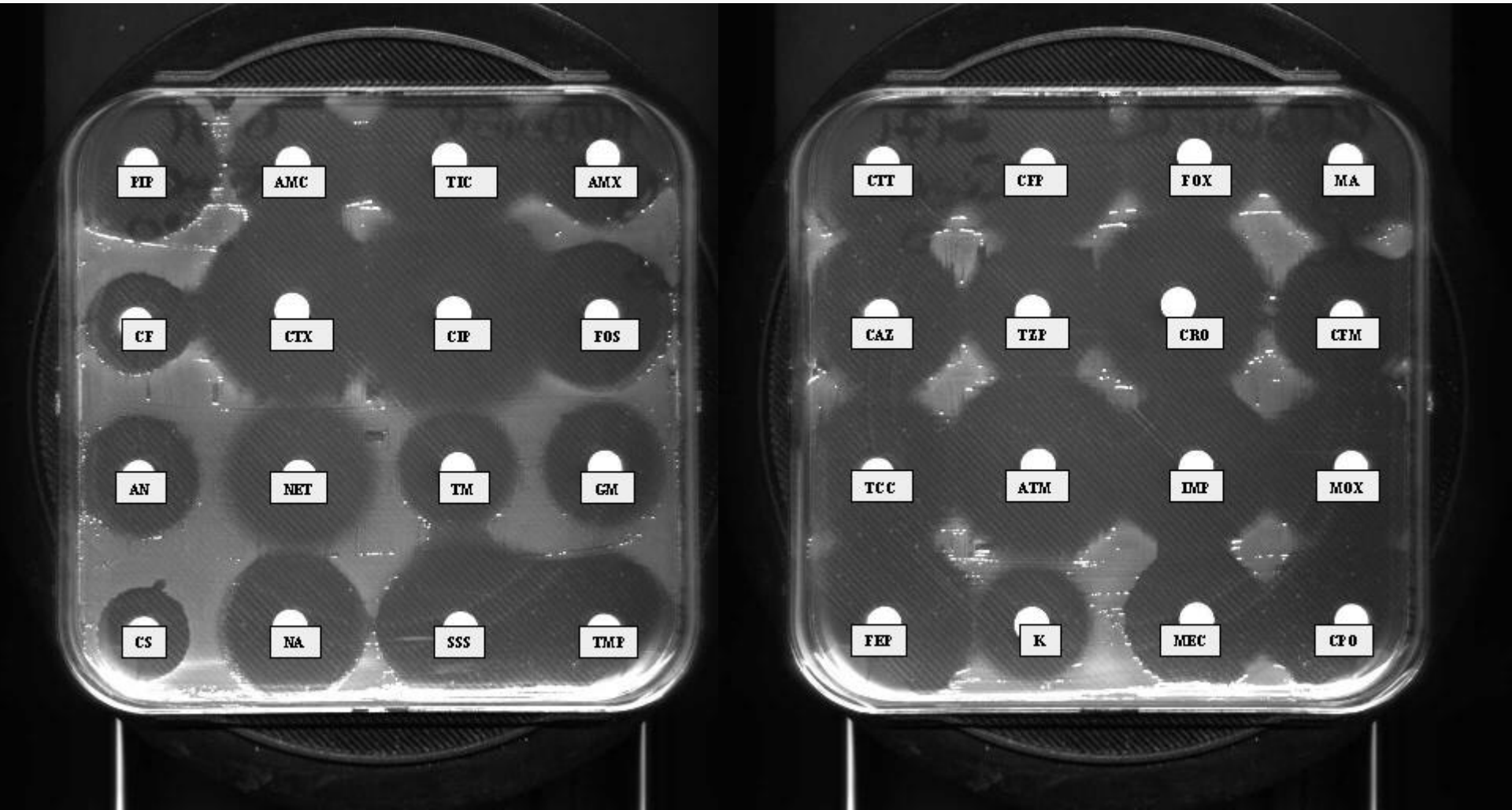
- Gradient method (E-test)



Enterobacteriaceae of groupe I

No intrinsic resistance to β-lactams

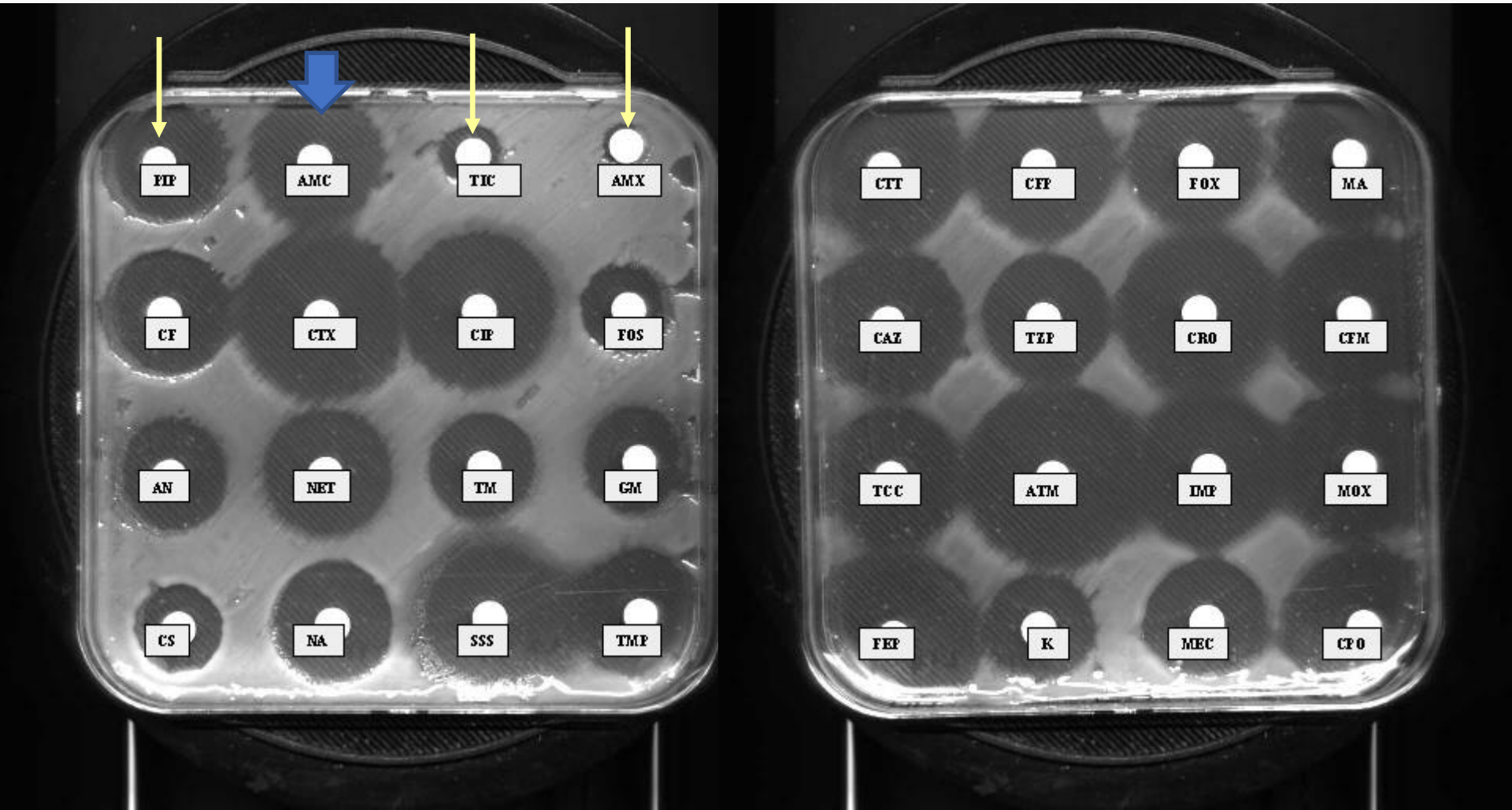
E. coli
P. mirabilis
Salmonella
Shigella



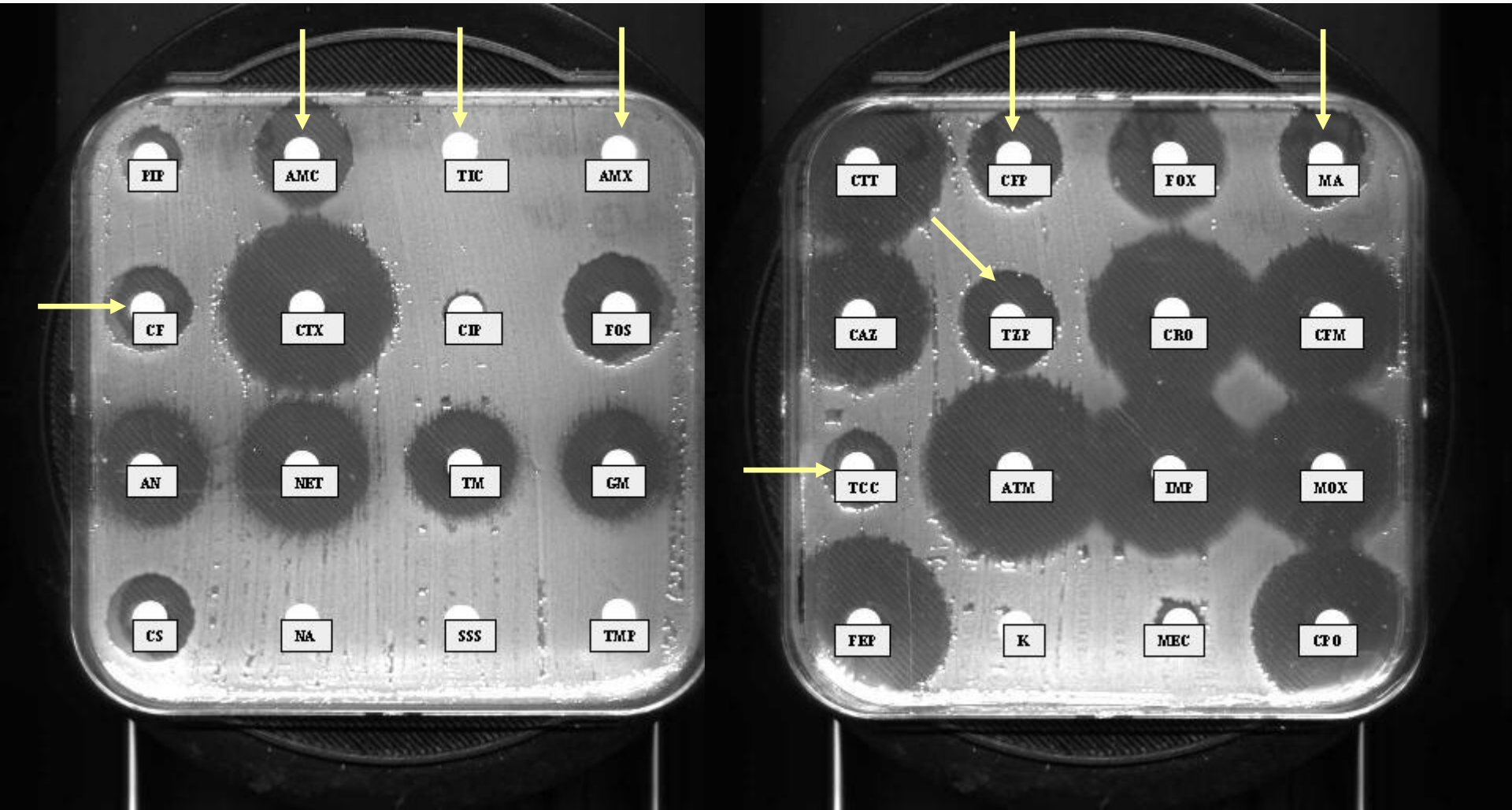
Enterobacteriaceae of groupe II
Chromosomic penicillinase

R to piperaciline, ticarcilline, amoxicilline

Klebsiella
Citrobacter koseri
Escherichia hermannii



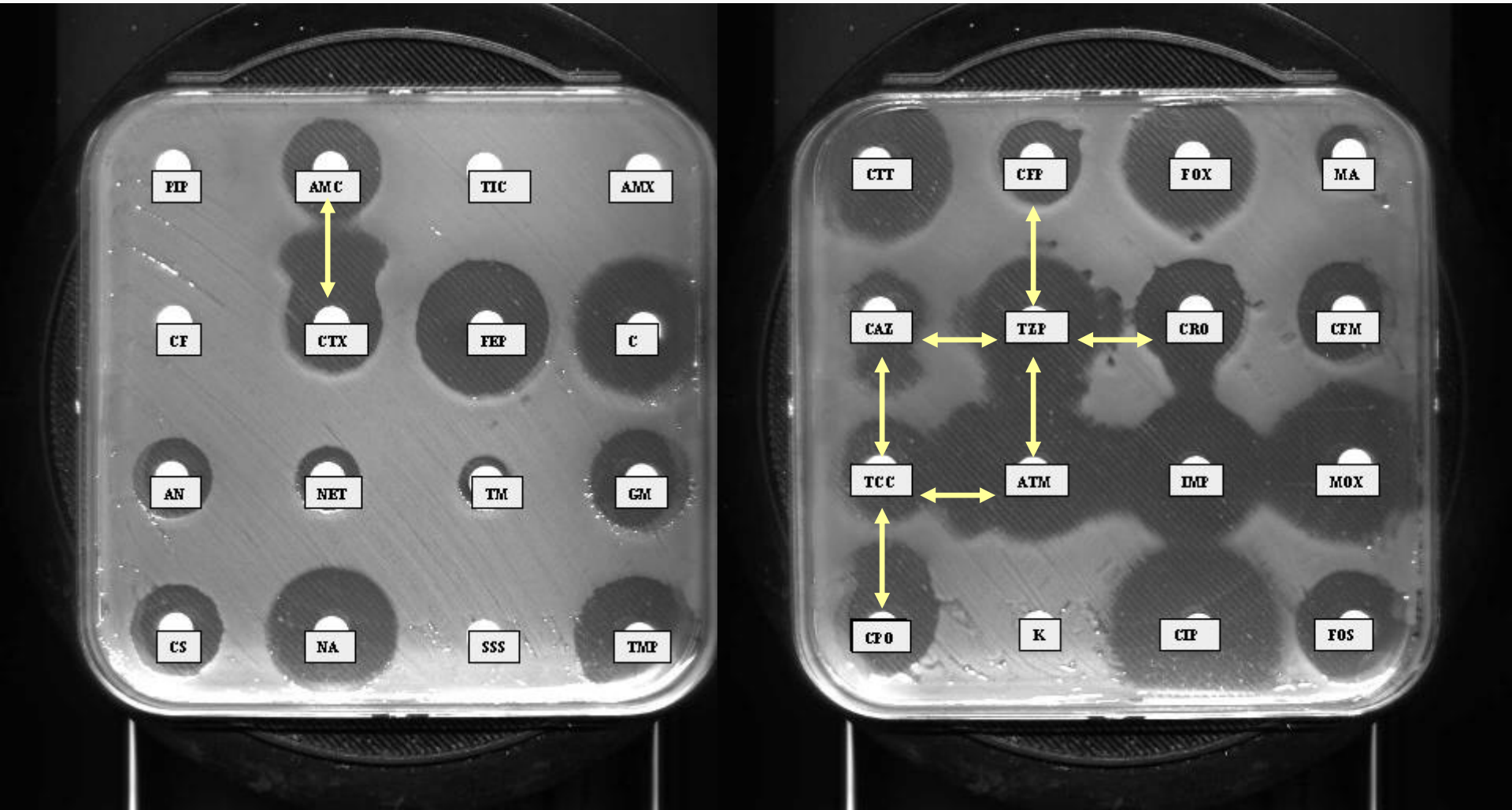
K. pneumoniae n° 39722283 : Hyperproduction of penicillinase



K. pneumoniae n° 39789032:

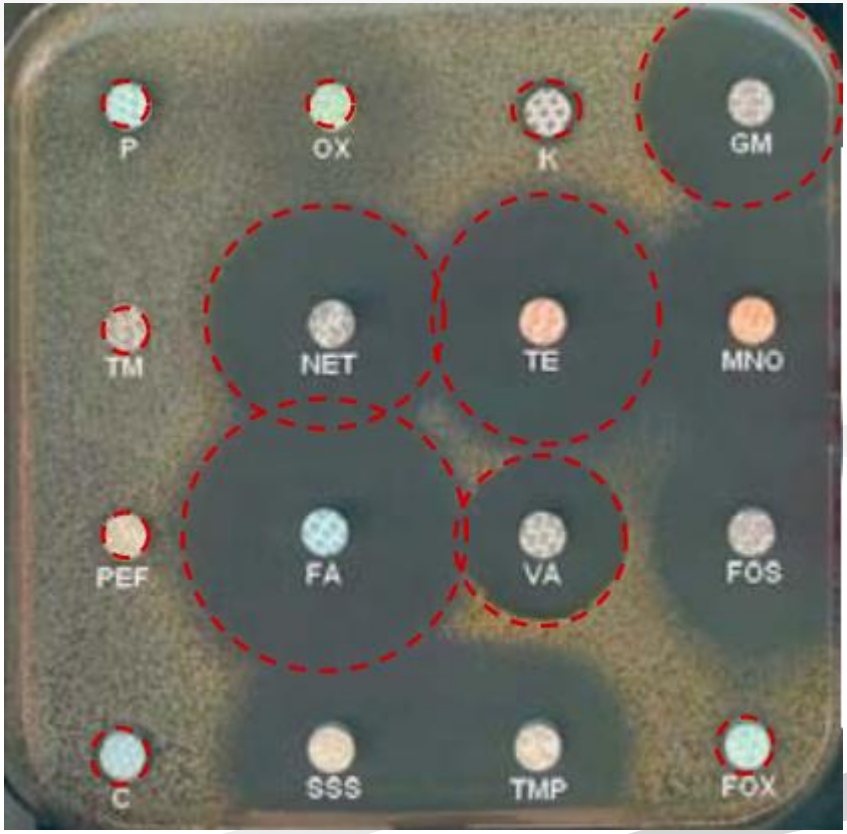
Extended-spectrum β-lactamases

Synergy +++

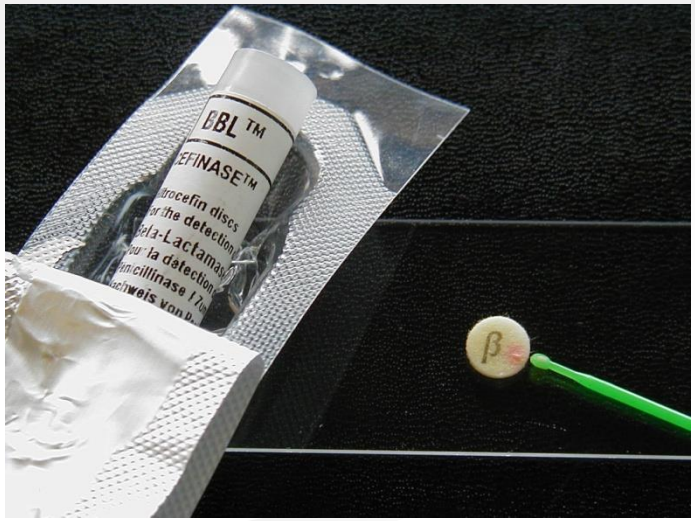


Staphylococcus aureus

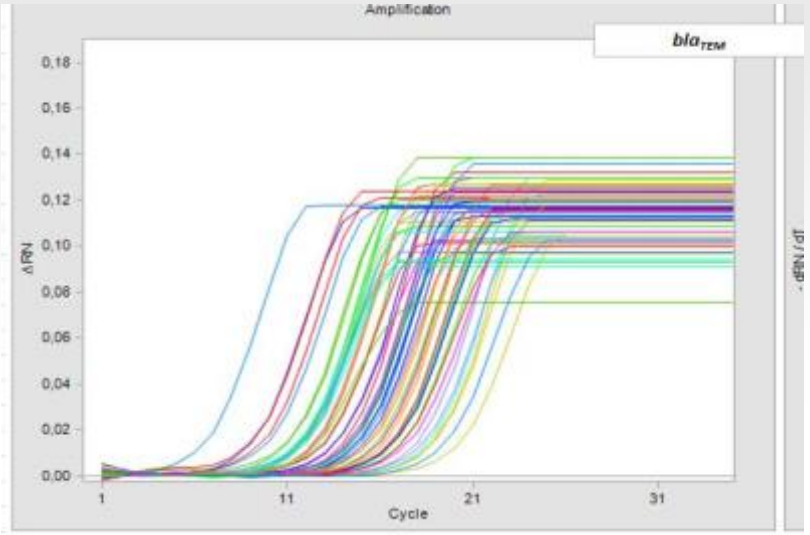
- Multidrug resistant strain:
  - Methicillin resistance
    - Oxacillin (Ox)
  - Fluroquinolone resistance
    - pefloxacin (pef)
  - Chloramphenicol resistance

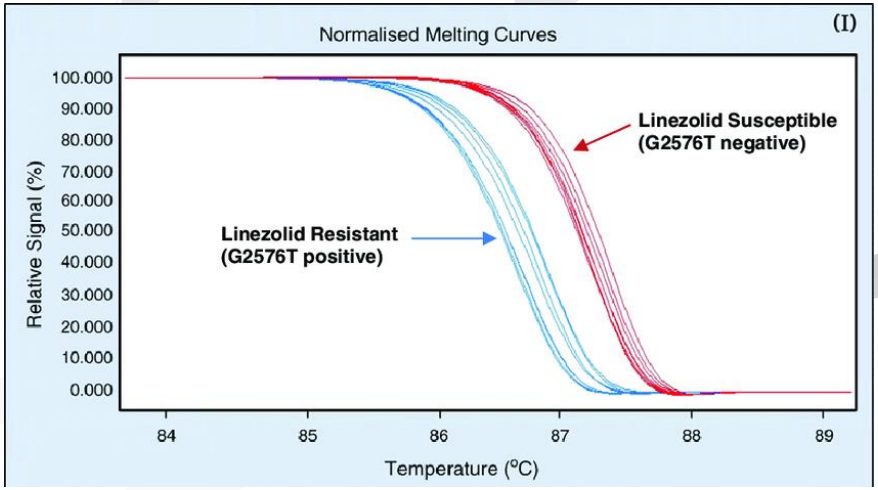


- By chromogenic test:
  - A Cefinase disc is impregnated with the chromogenic cephalosporin: nitrocefin.
  - nitrocefin exhibits a color change from yellow to red as the amide bond in the β-lactam ring is hydrolyzed by a β-lactamase.



- By PCR:
  - Using real time PCR machine
  - Detection of antibiotic resistance gene (example: beta lactamase gene)
  - Detection of mutation in the gene by analyzing the melting curve after PCR





Conclusions

- Knowing the mechanisms of antibiotic resistance is essential to treat infections
- Antibiotic resistance is associated with nearly 5 million deaths and killed at least 1.27 million people worldwide in 2019 (CDC, 2020)
- Among gram-positive spreading resistance to glycopeptides, Among gram-negative rods, spreading of extended-spectrum b-lactamases,and increased diffusion of carbapenemases
- World Health Organization estimates that by 2050, 10 million deaths worldwide could be due to infection linked to multidrug-resistant strains.

Thank you for your attention