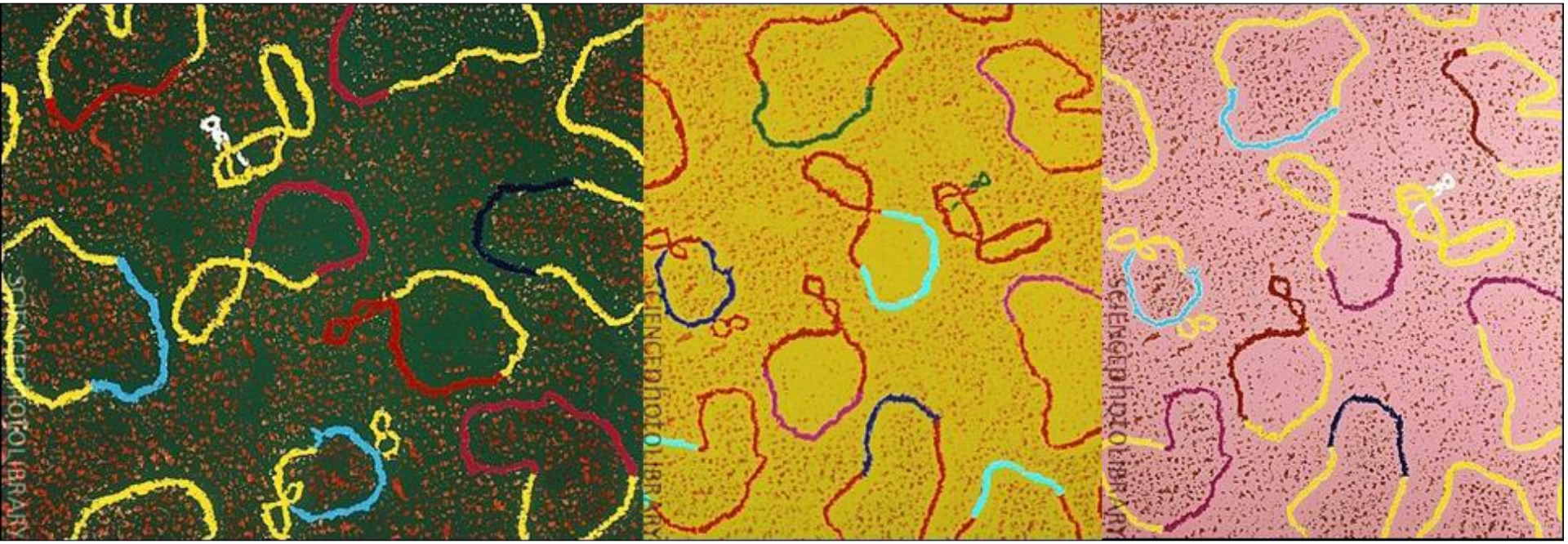
Antibiotics agents, drug resistance and spread of resistant bacteria



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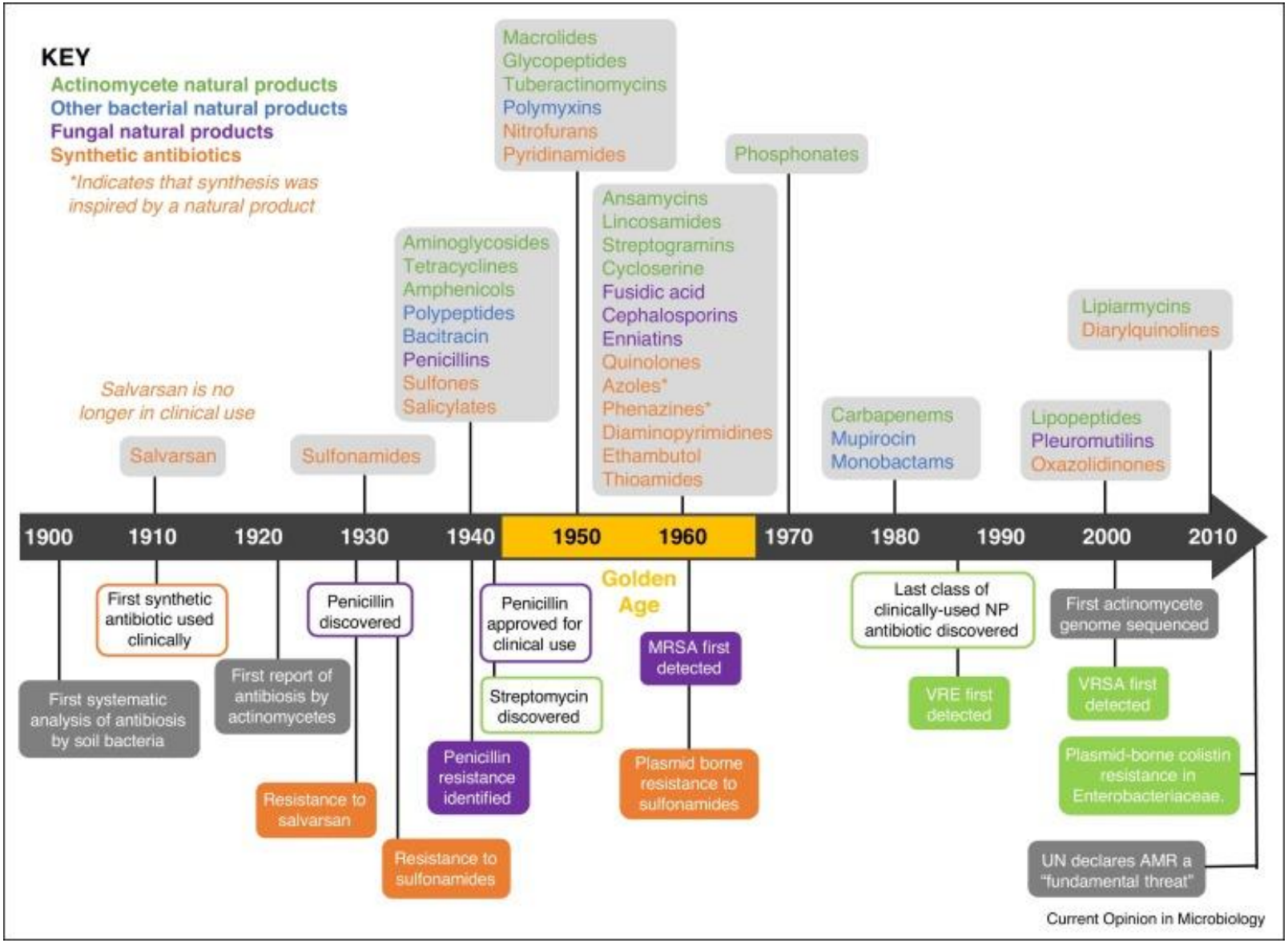
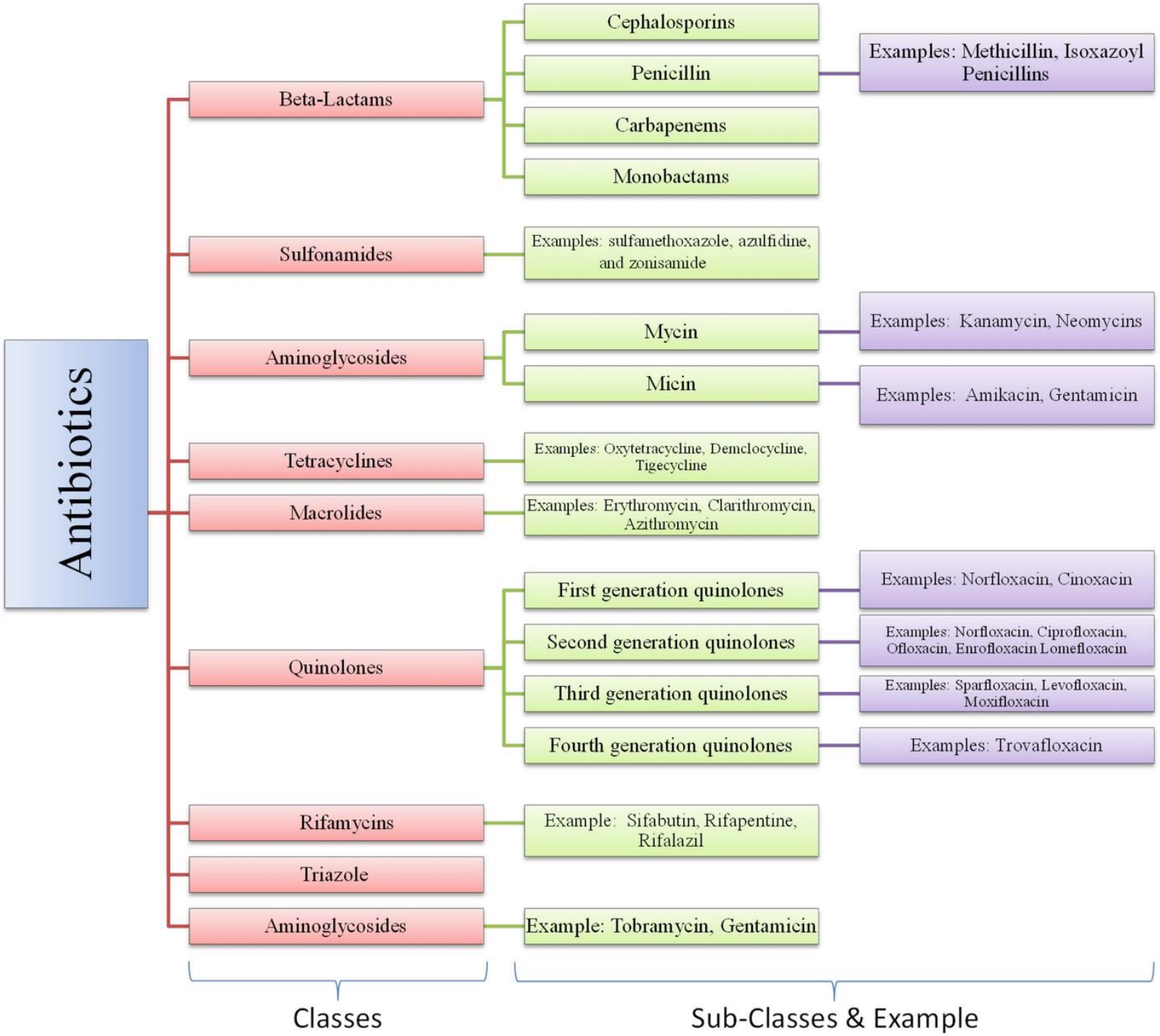
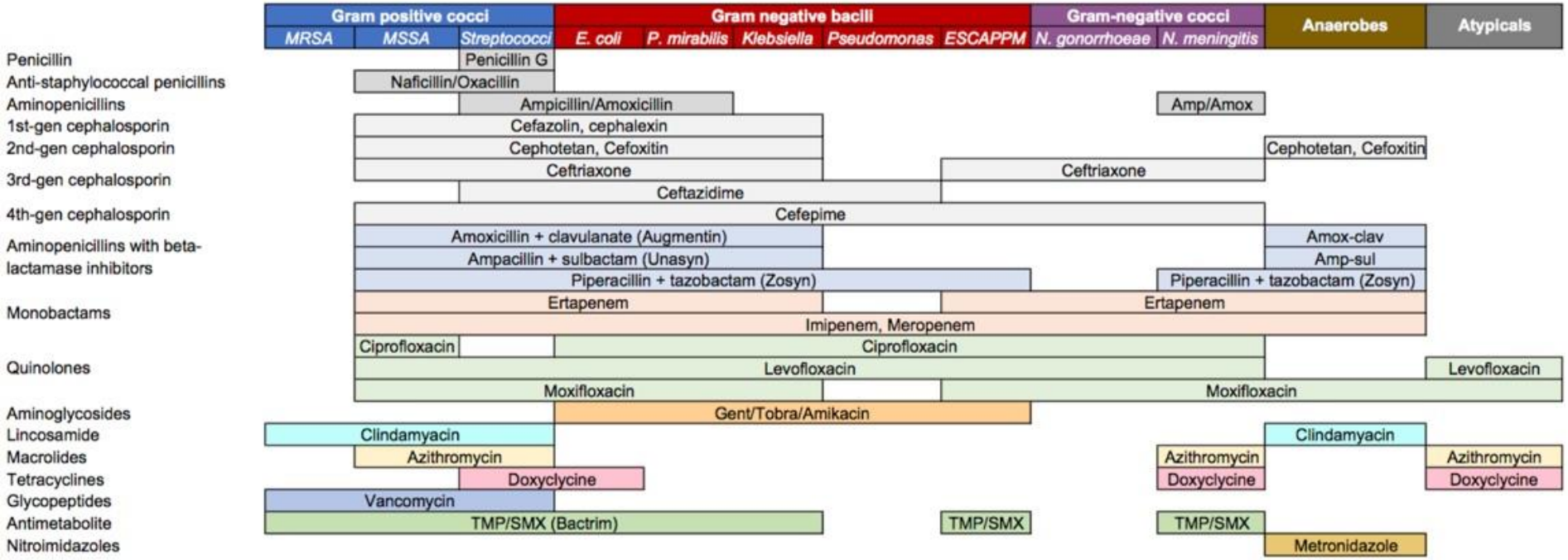
What are antibiotics?

Antibiotics kill bacteria or at least stop their growth

How many antibiotics do we have?

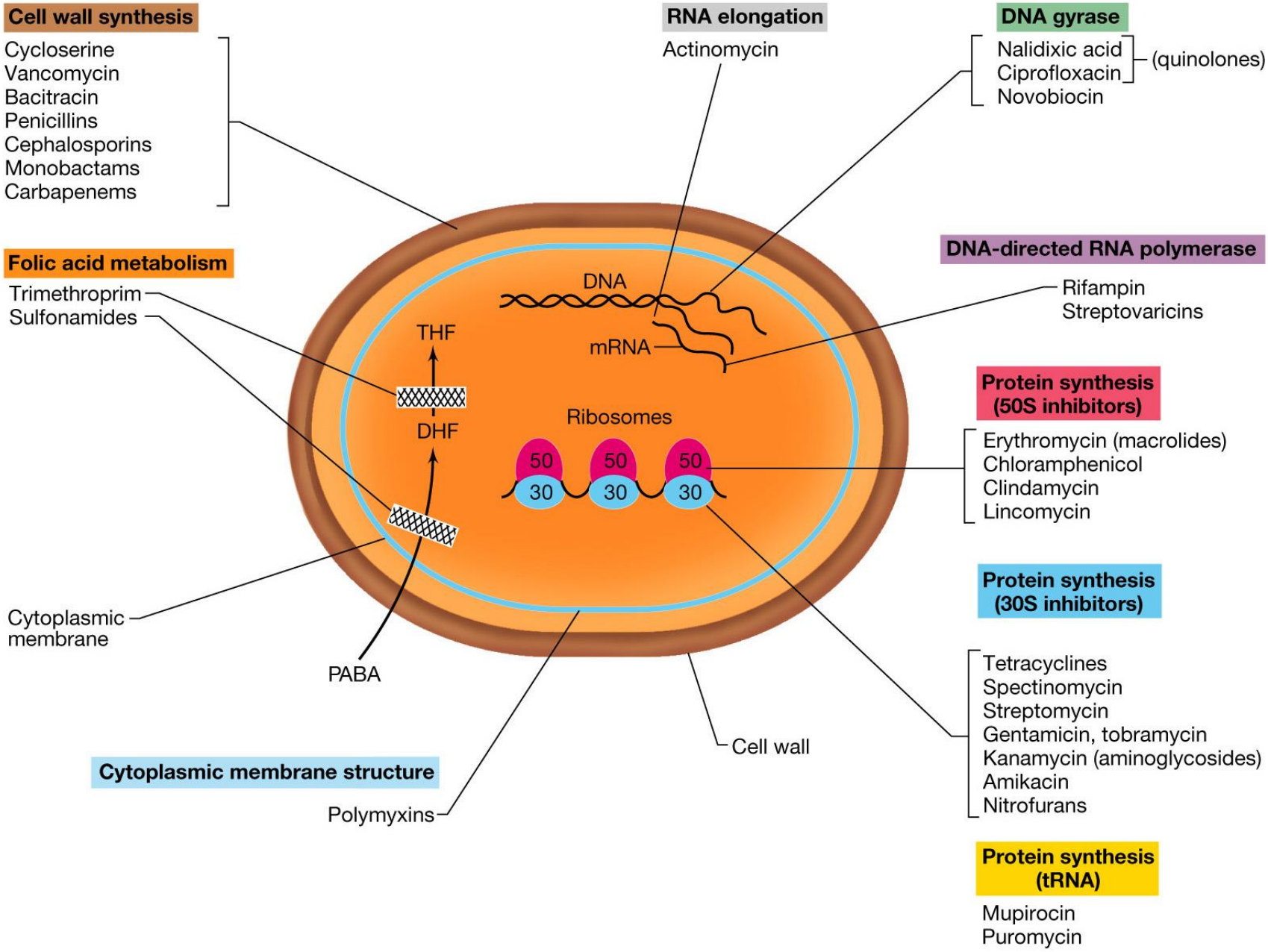


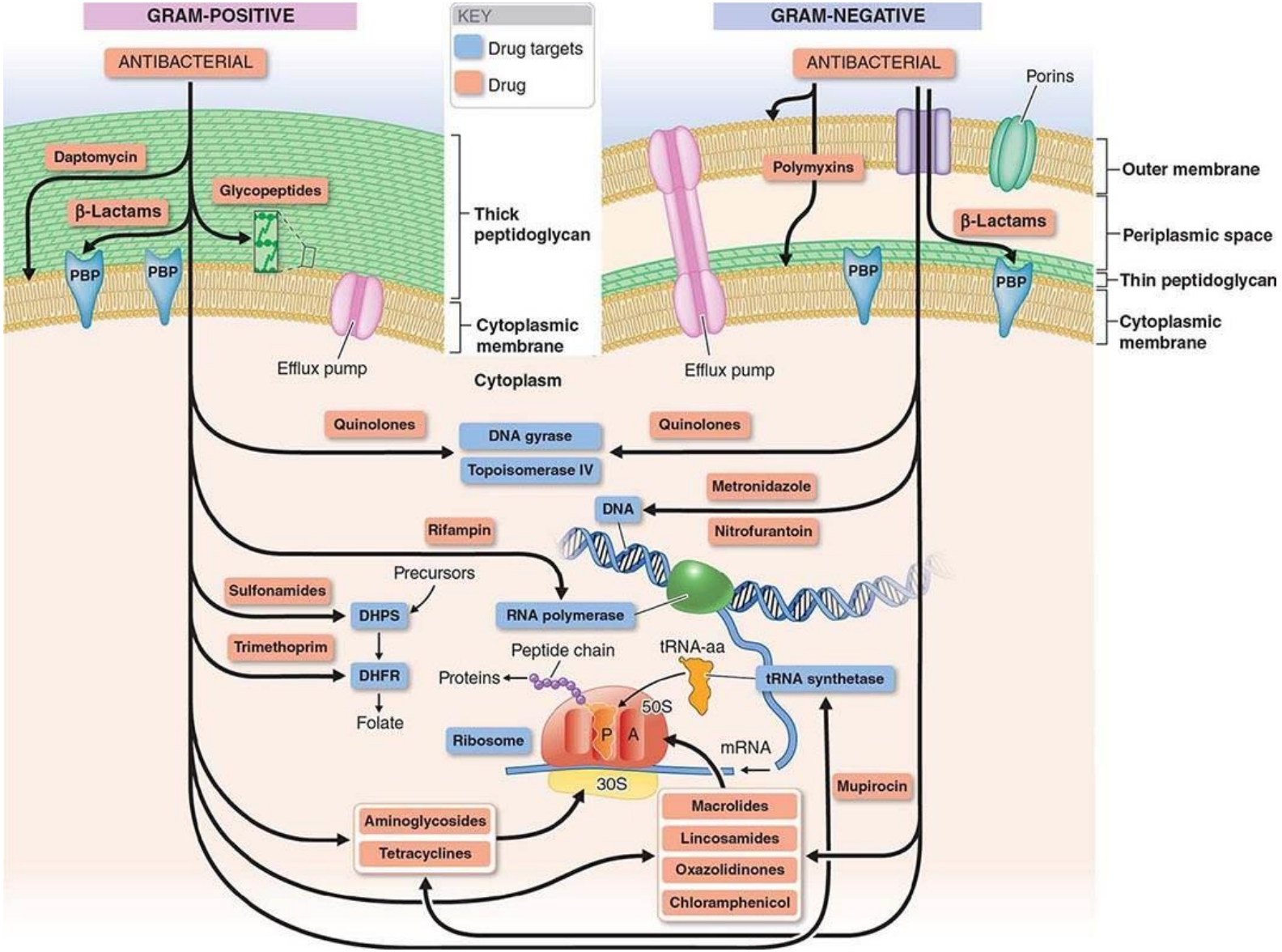

Antibiotics
Beta-Lactams
Cephalosporins
Penicillin
Examples: Methicillin, Isoxazoyl Penicillins
Carbapenems
Monobactams
Sulfonamides
Examples: sulfamethoxazole, azulfidine, and zonisamide
Aminoglycosides
Mycin
Examples: Kanamycin, Neomycins
Micin
Examples: Amikacin, Gentamicin
Tetracyclines
Examples: Oxytetracycline, Demclocycline, Tigecycline
Macrolides
Examples: Erythromycin, Clarithromycin, Azithromycin
Quinolones
First generation quinolones
Examples: Norfloxacin, Cinoxacin
Second generation quinolones
Examples: Norfloxacin, Ciprofloxacin, Ofloxacin, Enrofloxacin Lomefloxacin
Third generation quinolones
Examples: Sparfloxacin, Levofloxacin, Moxifloxacin
Fourth generation quinolones
Examples: Trovafloxacin
Rifamycins
Example: Sifabutin, Rifapentine, Rifalazil
Triazole
Aminoglycosides
Example: Tobramycin, Gentamicin
Classes
Sub-Classes & Example



See github.com/aetherist/antibiogram for details. For educational purposes only. TMP/SMX = Trimethoprim-sulfamethoxazole, MRSA = Methicillin-resistant Staphylococcus aureus, MSSA = Methicillin-sensitive Staphylococcus aureus, ESCAPPM = Enterobacter spp., Serratia spp., Citrobacter freundii, Aeromonas spp., Proteus spp., Providencia spp. and Morganella morganii.

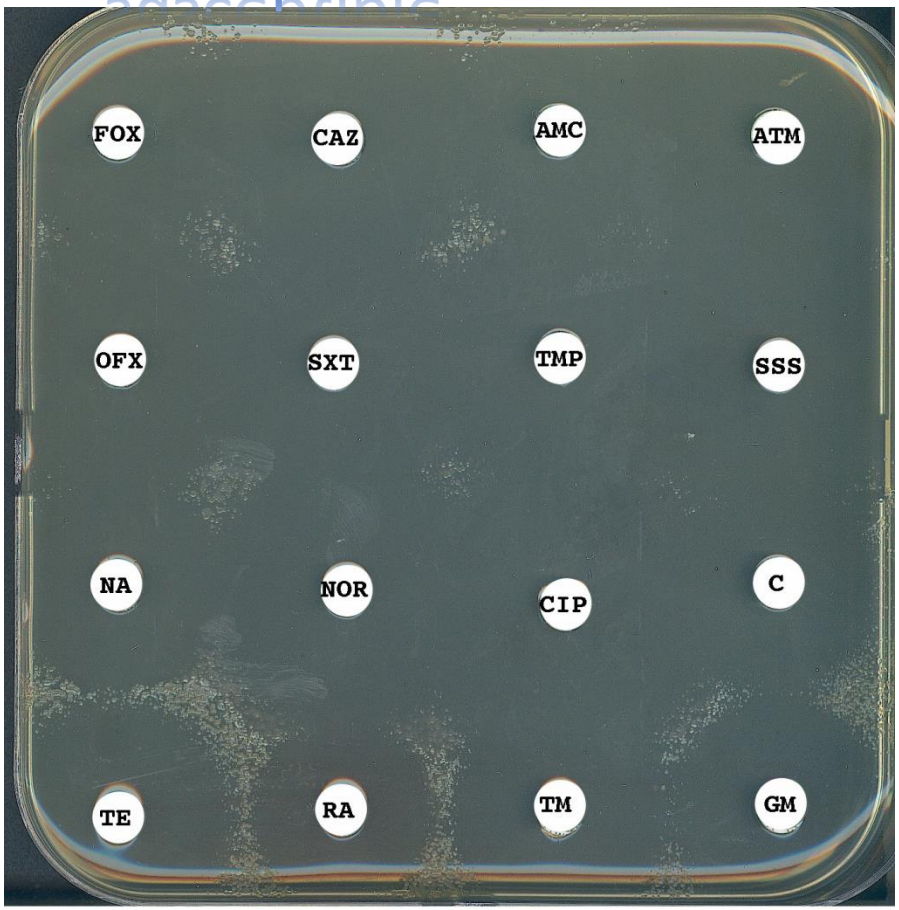
Antimicrobials target specific bacterial activities



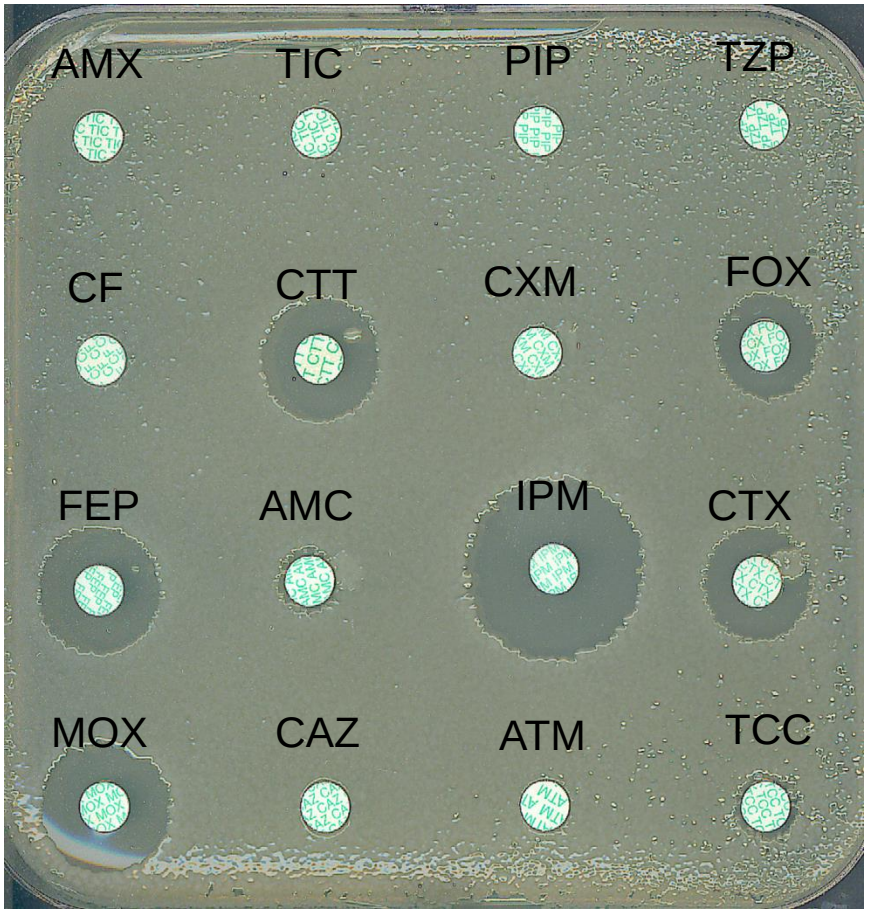

GRAM-POSITIVE
KEY
Drug targets
Drug
GRAM-NEGATIVE
ANTIBACTERIAL
ANTIBACTERIAL
Porins
Daptomycin
Glycopeptides
β-Lactams
PBP
PBP
Thick peptidoglycan
Cytoplasmic membrane
Efflux pump
Polymyxins
β-Lactams
PBP
PBP
Outer membrane
Periplasmic space
Thin peptidoglycan
Cytoplasmic membrane
Efflux pump
Cytoplasm
Quinolones
DNA gyrase
Topoisomerase IV
Quinolones
Metronidazole
DNA
Rifampin
Nitrofurantoin
Precursors
Sulfonamides
DHPS
RNA polymerase
Trimethoprim
DHFR
Folate
Peptide chain
tRNA-aa
Proteins
tRNA synthetase
50S
Ribosome
P
A
mRNA
30S
Mupirocin
Aminoglycosides
Tetracyclines
Macrolides
Lincosamides
Oxazolidinones
Chloramphenicol

Antimicrobial Resistance

Escherichia coli susceptible



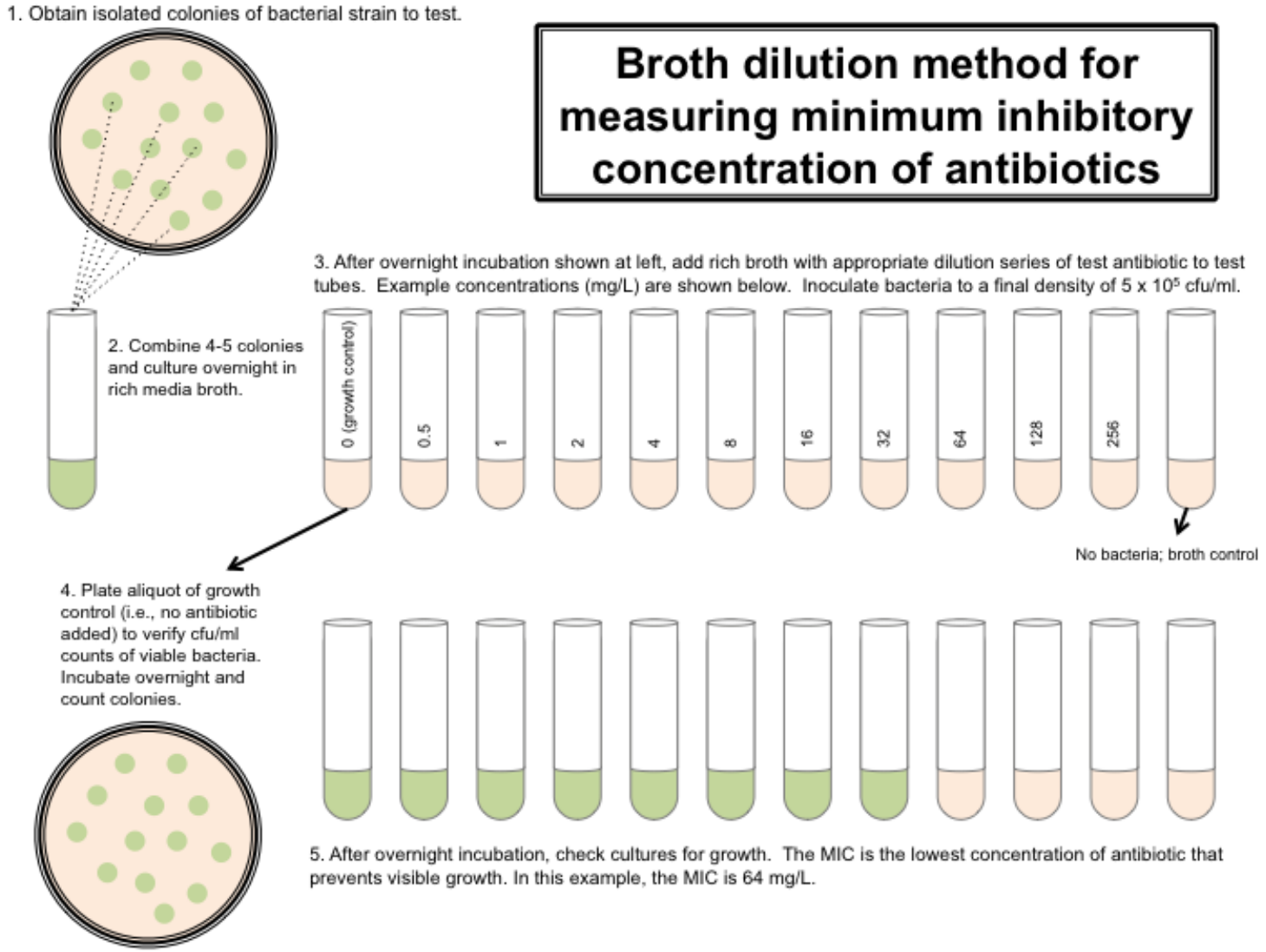
Escherichia coli resistant



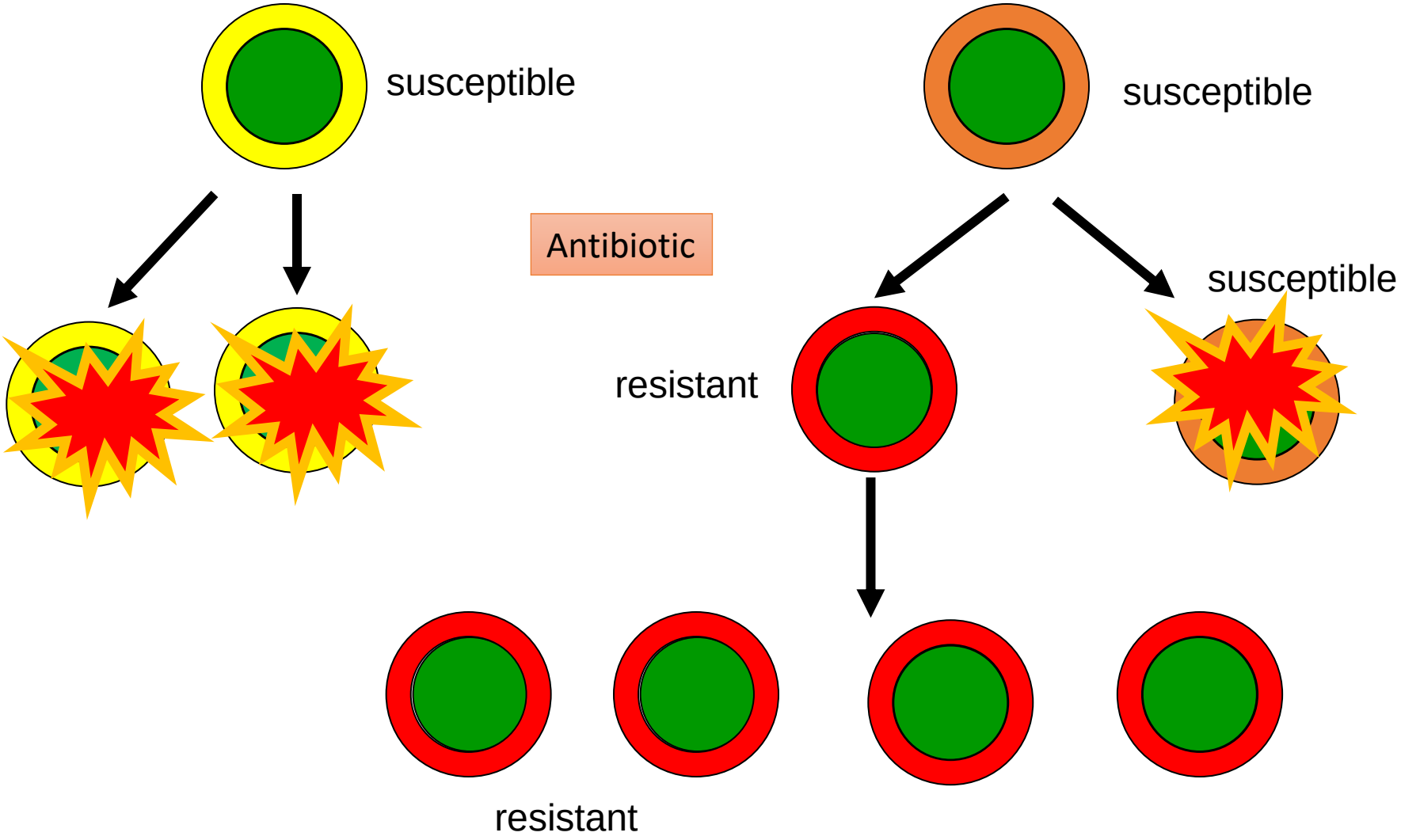
Antibiogram

AMX : amoxicillin CAZ : ceftazidime AMC : amoxicilline + clav FEP : cefepime
TCC : ticarcillie + clav TZP : piperacillin + tazobactam CTX : cefotaxime CS : colistine
CF : cefalotine TIC : ticarcillin TM : tobramycin AN : amikacine
GM : gentamicin OFX : ofloxacin CIP : ciprofloxacin IPM : imipenem

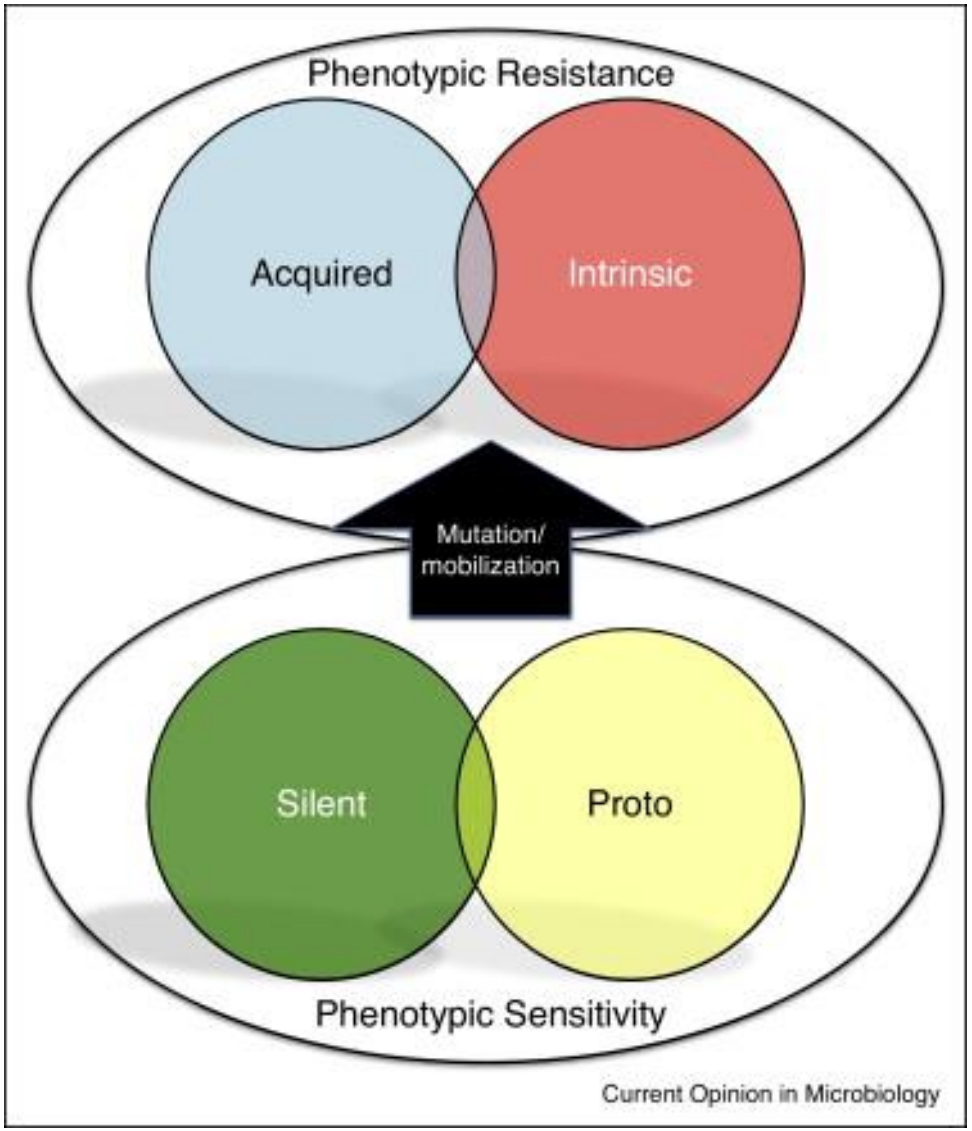
Minimal Inhibent concentration(MIC)



Bacteria point of view: a question of life or death



Resistome: all resistance genes, known and unknown that circulate on the planet



The antibiotic resistome: what's new? Julie Ann Perry, Erin Louise Westman, Gerard D Wright Current Opinion in Microbiology, Volume 21, 2014, 45–50

Table 1. Intrinsic resistance in Enterobacteriaceae. Enterobacteriaceae are also intrinsically resistant to benzylpenicillin, glycopeptides, fusidic acid, macrolides (with some exceptions[1]), lincosamides, streptogramins, rifampicin, daptomycin and linezolid.

Rule no.	Organisms	Ampicillin	Amoxicillin-Clavulanic acid	Ampicillin-sulbactam	Ticarcillin	Cefazolin, Cefalotin Cefalexin, Cefadroxil	Cefoxitin[2]	Cefuroxime	Tetracyclines	Tigecycline	Polymyxin B, Colistin	Nitrofurantoin
1.1	*Citrobacter koseri,Citrobacter amalonaticus*[3]	R			R							
1.2	*Citrobacter freundii*[4]	R	R	R		R	R					
1.3	*Enterobacter cloacae* complex	R	R	R		R	R					
1.4	*Enterobacter aerogenes*	R	R	R		R	R					
1.5	*Escherichia hermannii*	R			R							
1.6	*Hafnia alvei*	R	R	R		R	R					
1.7	*Klebsiella pneumoniae*	R			R							
1.8	*Klebsiella oxytoca*	R			R							
1.9	*Morganella morganii*	R	R	R		R			R		R	R
1.10	*Proteus mirabilis*								R	R	R	R
1.11	*Proteus penneri*	R				R		R	R	R	R	R
1.12	*Proteus vulgaris*	R				R		R	R	R	R	R
1.13	*Providencia rettgeri*	R	R	R		R		R	R	R	R	R
1.14	*Providencia stuartii*	R	R	R		R		R	R	R	R	R
1.15	*Raoultella* spp.	R			R							
1.16	*Serratia marcescens*	R	R	R		R	R	R	R[5]		R	R
1.17	*Yersinia enterocolitica*	R	R	R	R	R	R					
1.18	*Yersinia pseudotuberculosis*										R	

R = resistant

[1] Azithromycin is effective *in vivo* for the treatment of typhoid fever and erythromycin may be used to treat travellers' diarrhoea.

Table 4. Intrinsic resistance in Gram-positive bacteria. Gram-positive bacteria are also intrinsically resistant to aztreonam, temocillin, polymyxin B/colistin and nalidixic acid

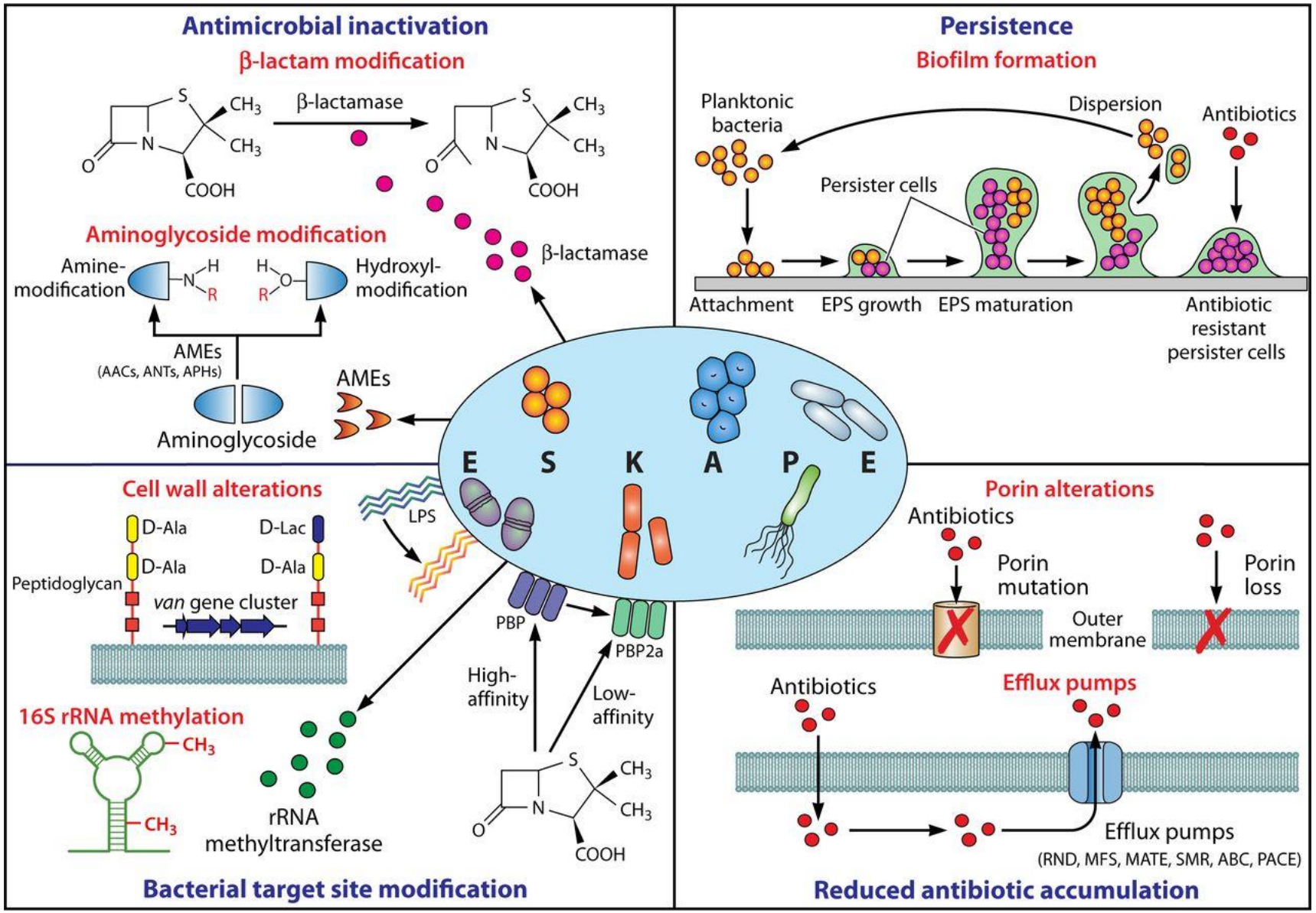
Rule no.	Organisms	Fusidic acid	Ceftazidime	Cephalosporins (except ceftazidime)	Aminoglycosides	Macrolides	Clindamycin	Quinupristin-dalfopristin	Vancomycin	Teicoplanin	Fosfomycin	Novobiocin	Sulfonamides
4.1	*Staphylococcus saprophyticus*	R	R								R	R	
4.2	*Staphylococcus cohnii,*		R									R	
4.3	*Staphylococcus xylosus*		R									R	
4.4	*Staphylococcus capitis*		R								R		
4.5	Other coagulase-negative staphylococci and *Staphylococcus aureus*		R										
4.6	*Streptococcus* spp.	R	R		R[1]								
4.7	*Enterococcus faecalis*	R	R	R	R[1]	R	R	R					R
4.8	*Enterococcus gallinarum, Enterococcus casseliflavus*	R	R	R	R[1]	R	R	R	R				R
4.9	*Enterococcus faecium*	R	R	R	R[1,2]	R							R
4.10	*Corynebacterium* spp.										R		
4.11	*Listeria monocytogenes*		R	R									
4.12	*Leuconostoc* spp., *Pediococcus* spp.								R	R			
4.13	*Lactobacillus* spp. (*L. casei, L. casei* var. *rhamnosus*)								R	R			
4.14	*Clostridium ramosum, Clostridium innocuum*								R				

R = resistant

[1] Low-level resistance (LLR) to aminoglycosides. Combinations of aminoglycosides with cell wall inhibitors (penicillins and glycopeptides) are synergistic and bactericidal against isolates that are susceptible to cell wall inhibitors and do not display high-level resistance to aminoglycosides.

[2] In addition to LLR to aminoglycosides, *Enterococcus faecium* produces a chromosomal AAC(6')-I enzyme that is responsible for the loss of synergism between aminoglycosides (except gentamicin, amikacin and streptomycin) and penicillins or glycopeptides.

antimicrobial resistance



David M. P. De Oliveira et al. Clin. Microbiol. Rev. 2020; doi:10.1128/CMR.00181-19

Progenitors

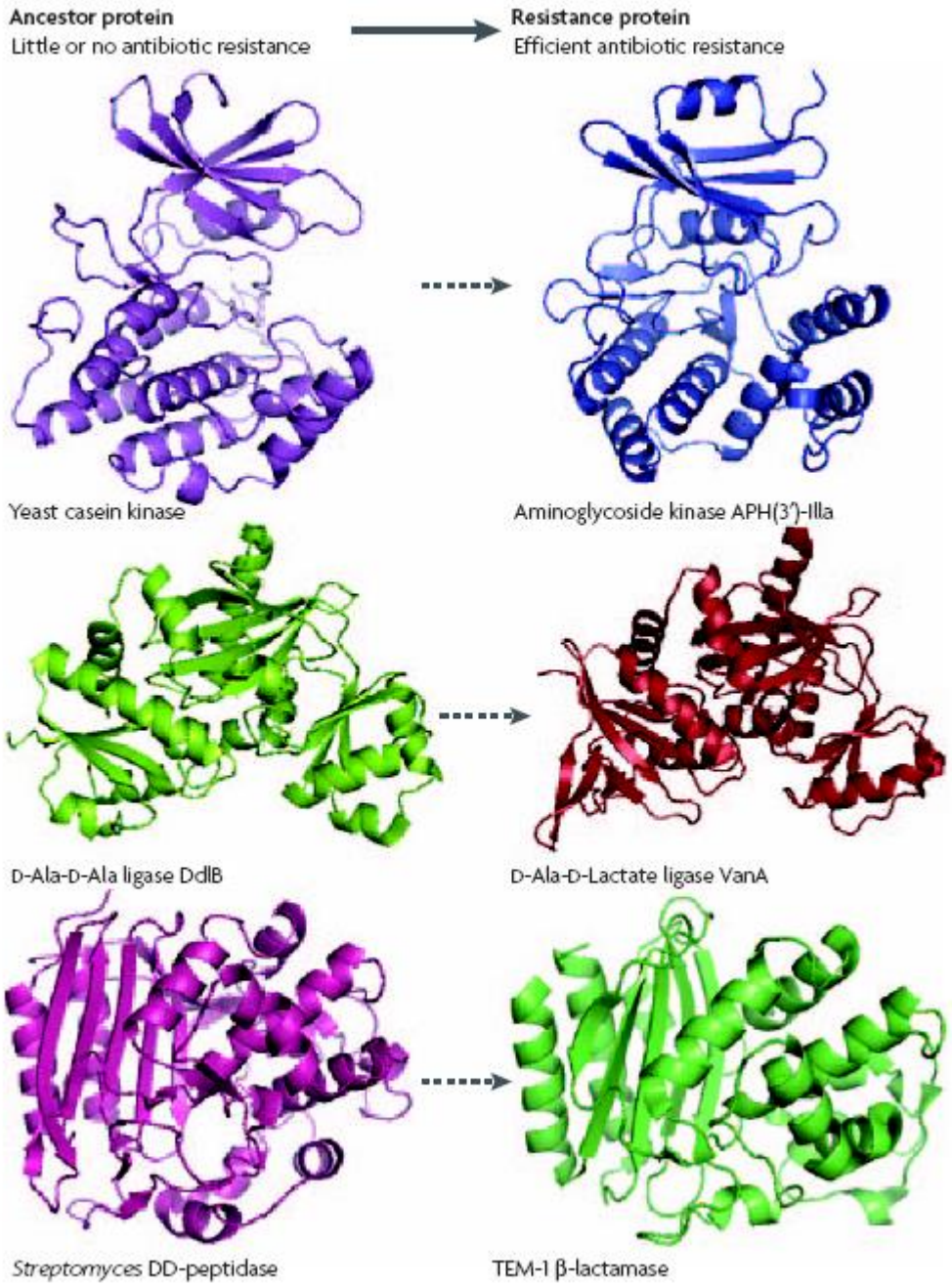


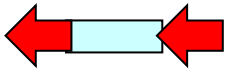
Figure 6 | **Evolution of antibiotic resistance proteins.** Protein structure and

The antibiotic resistome
Wright, G.D. Nature Reviews Microbiology, 5:175, 2007

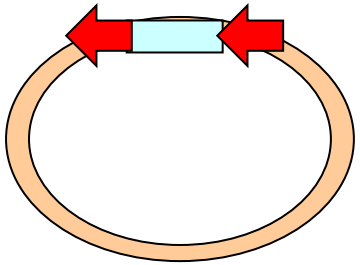
Acquired antimicrobial resistance

- genes

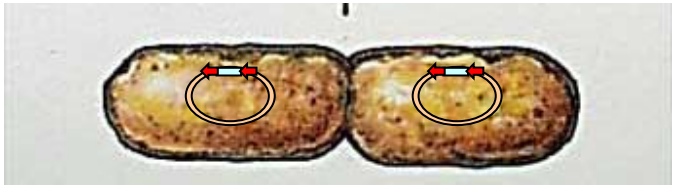
- transposons



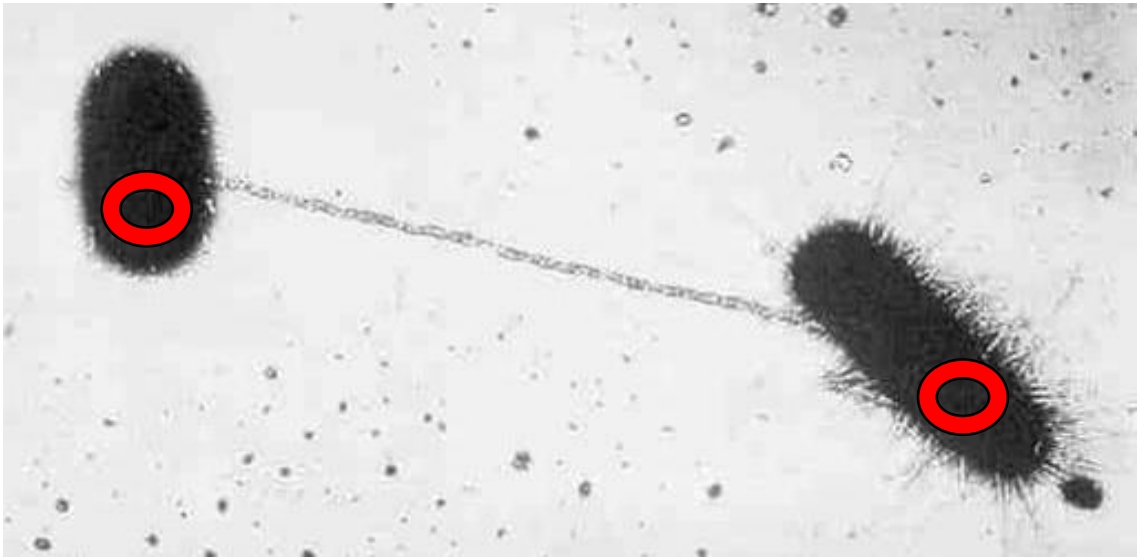
- plasmids



- bacterial clones

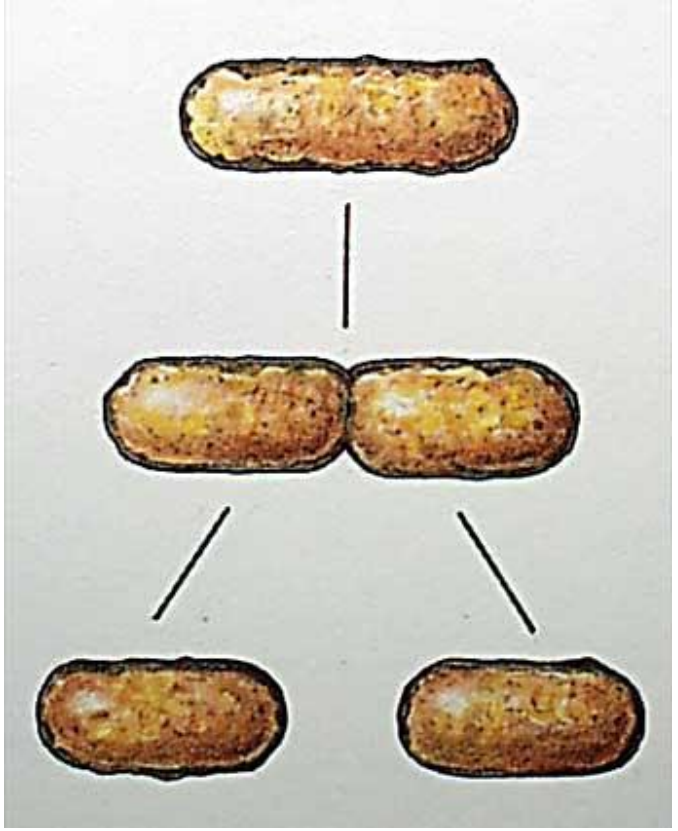


Horizontal



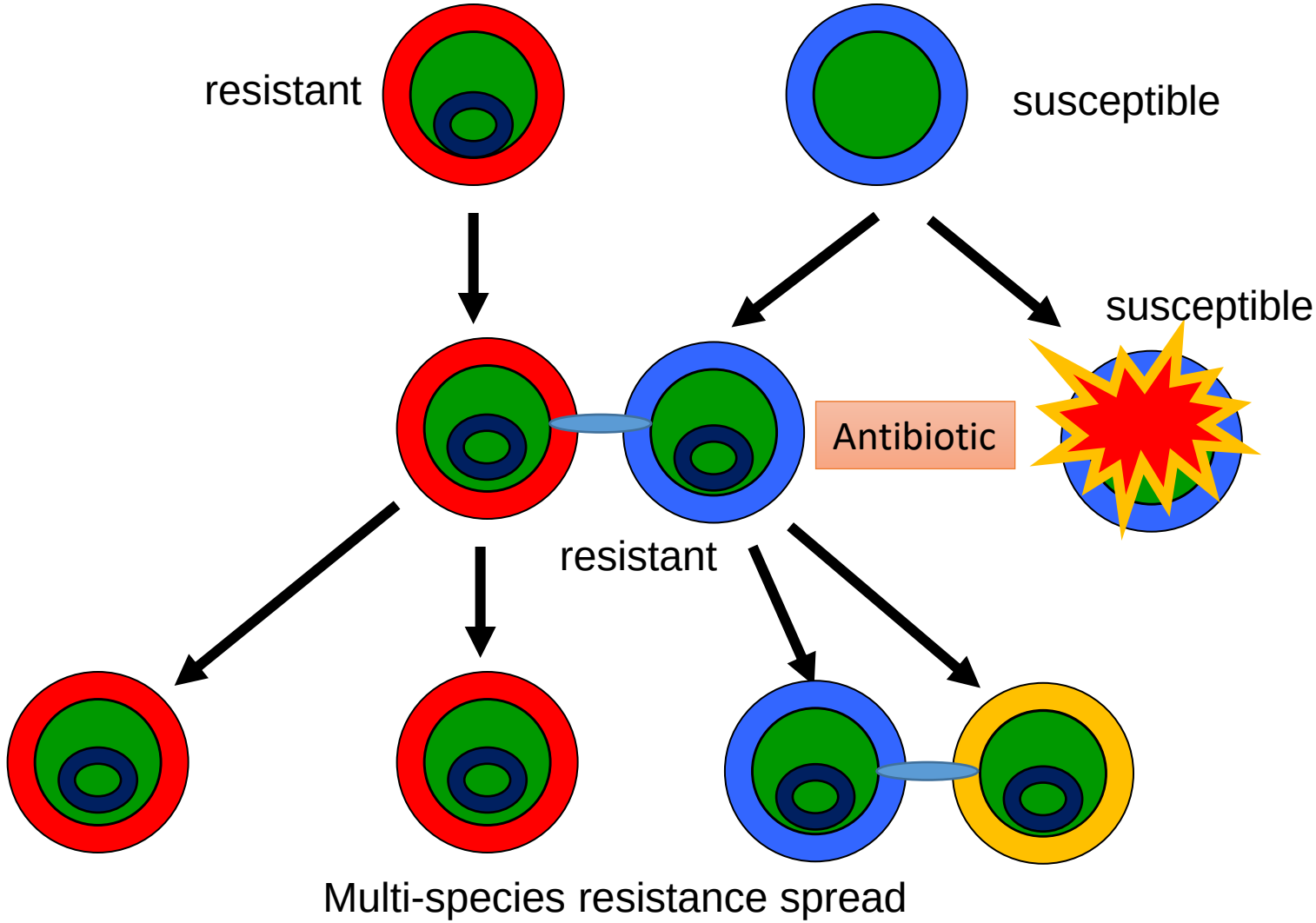
Transmission across species boundaries

Vertical

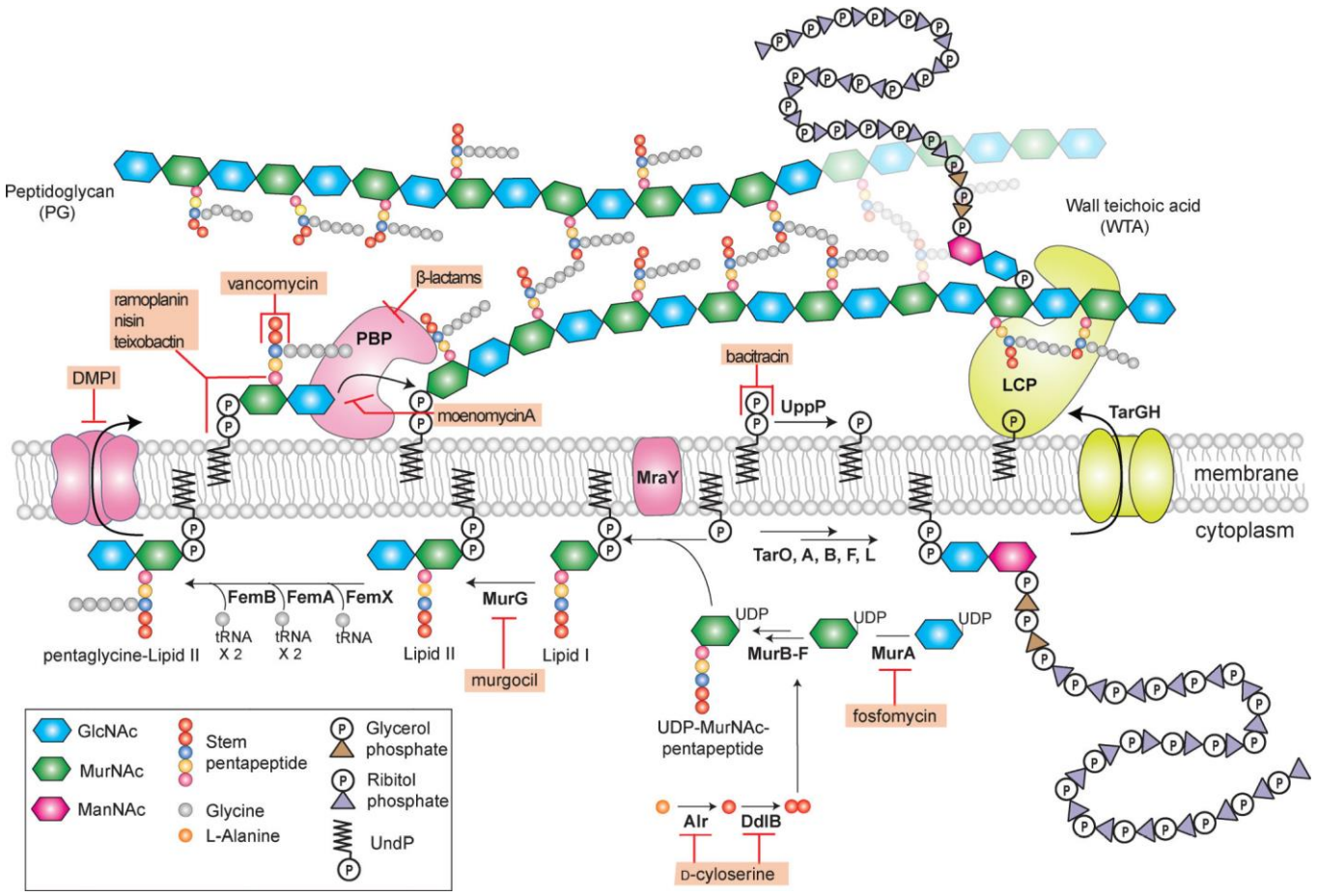


Clonal transmission

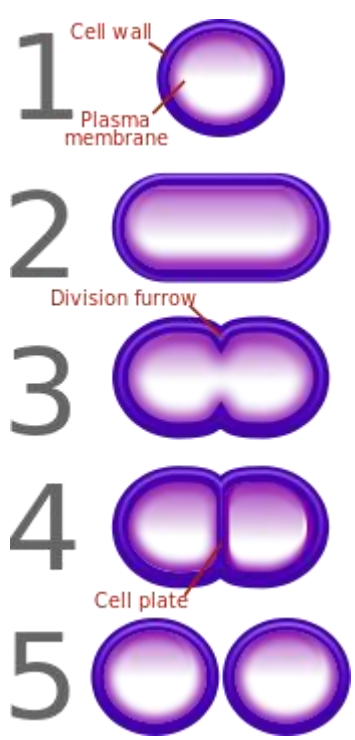
Impact of horizontal transmission

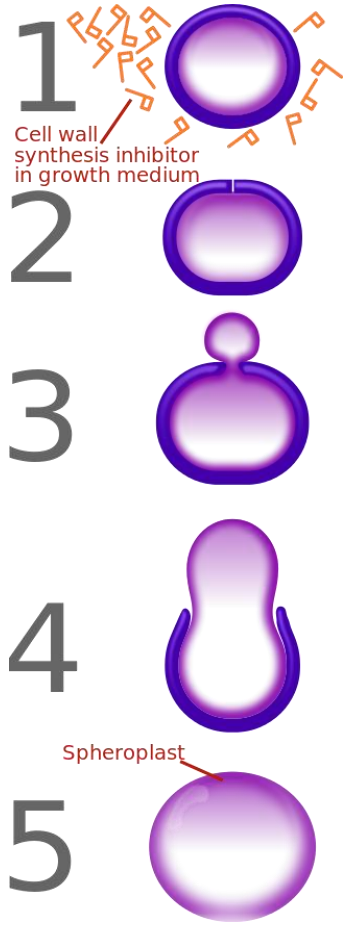


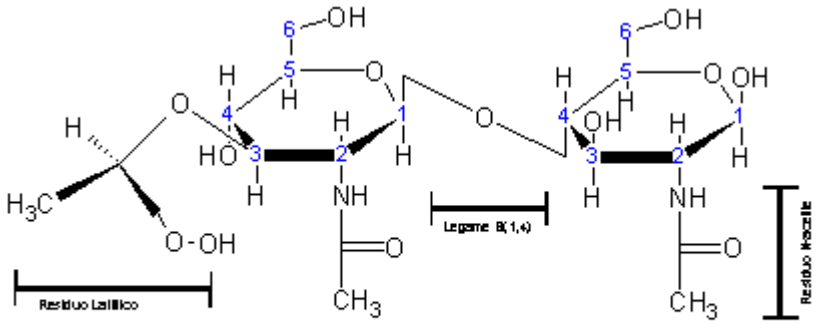
Antibiotics that inhibits the synthesis of peptidoglycan

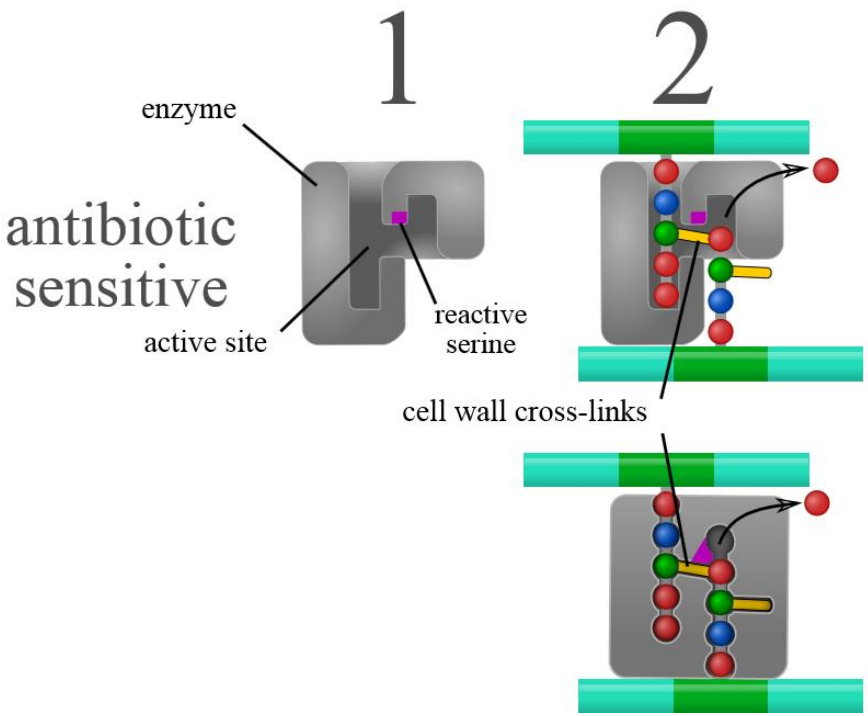


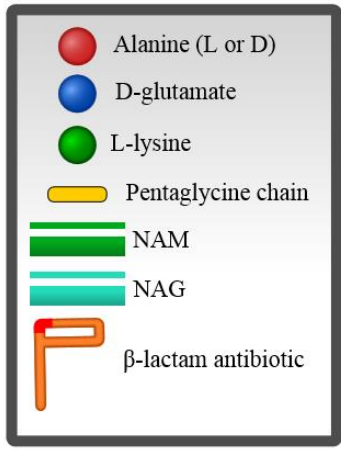
Beta-lactams


1
Cell wall
Plasma membrane
2
Division furrow
3
4
Cell plate
5


1
Cell wall synthesis inhibitor in growth medium
2
3
4
Spheroplast
5

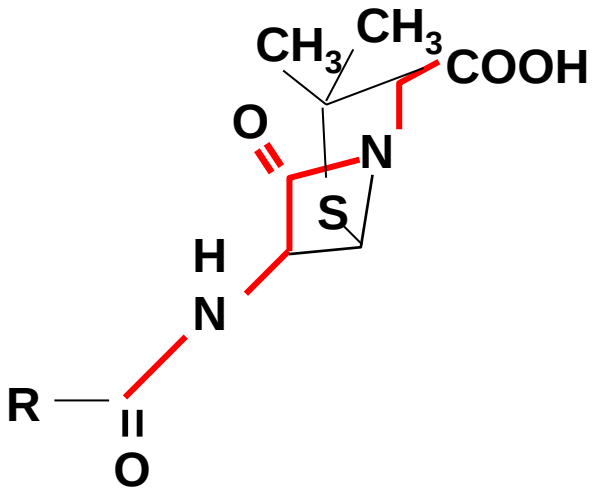

NAM
NAG


1
2
enzyme
antibiotic sensitive
active site
reactive serine
cell wall cross-links

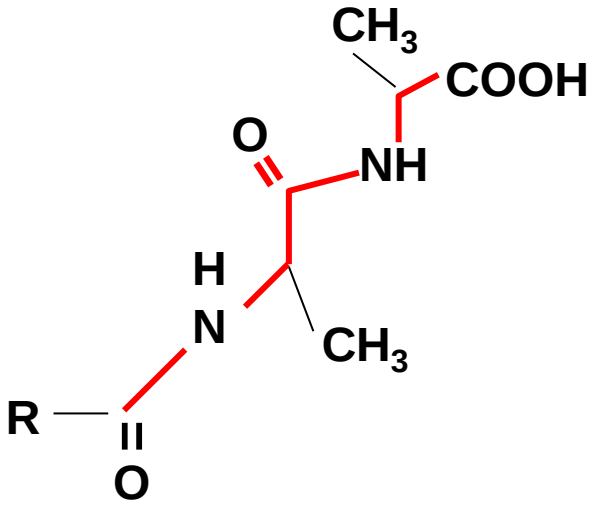

Alanine (L or D)
D-glutamate
L-lysine
Pentaglycine chain
NAM
NAG
β-lactam antibiotic

β-LACTAMS

MECHANISM OF ACTION
INHIBITION OF PEPTIDOGLYCAN SYNTHESIS AT THE LEVEL OF THE FORMATION OF THE TRANSPEPTIDATION BOND BETWEEN PENTAPEPTIDES OF TWO ADJACENT CHAINS
BACTERICIDES

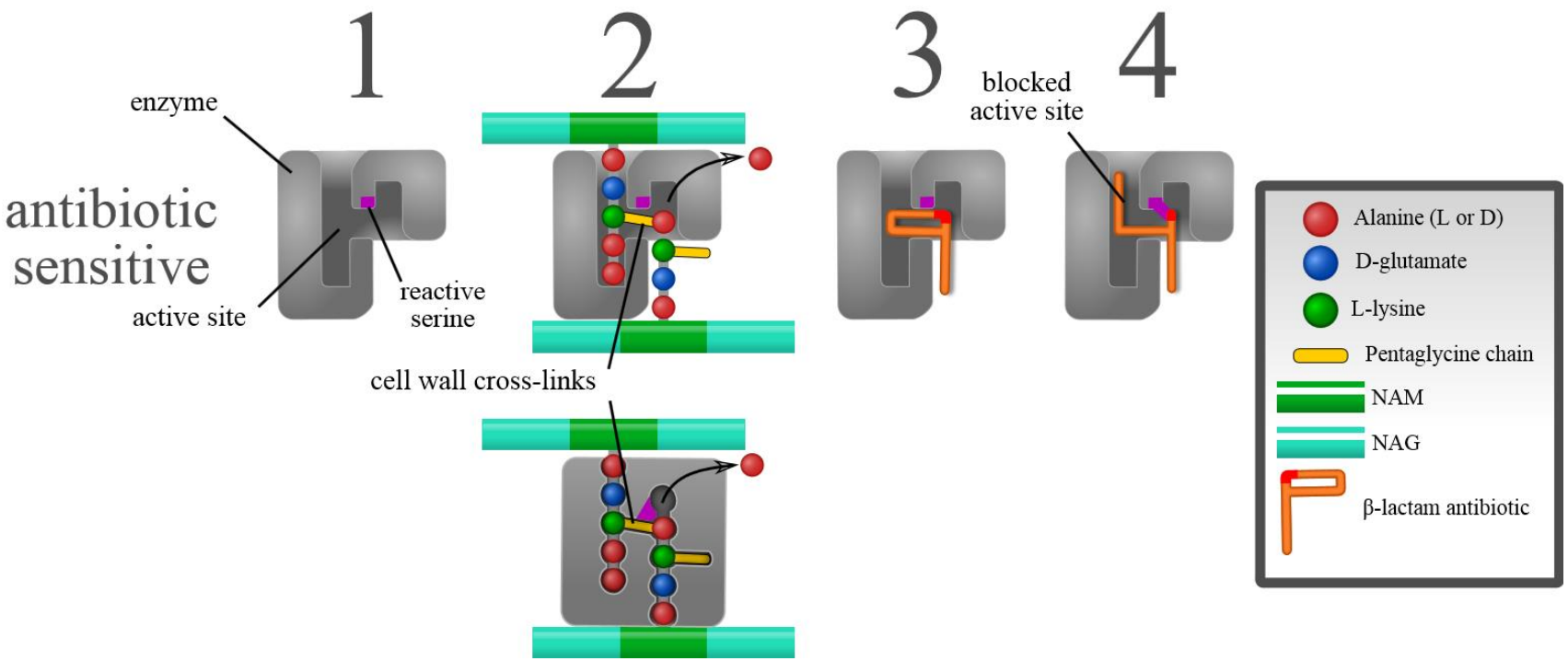


β - LACTAM

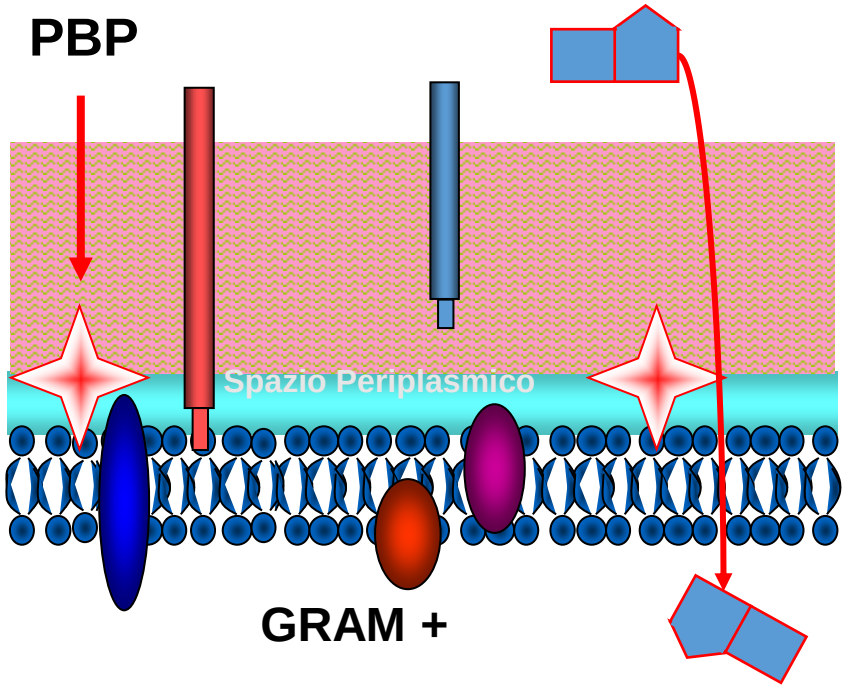


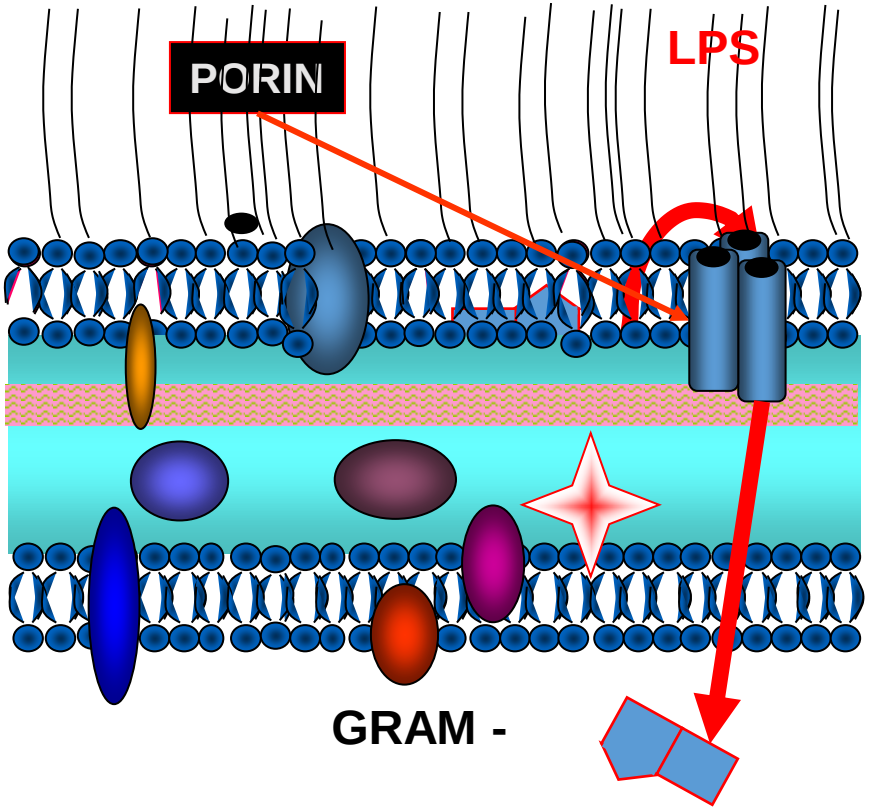
D-ALA

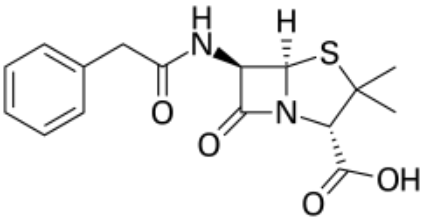
β-lactams irreversibly bind to the active site of PBP, making transpeptidation impossible. This makes the peptidoglycan wall of the bacterium incomplete and brittle



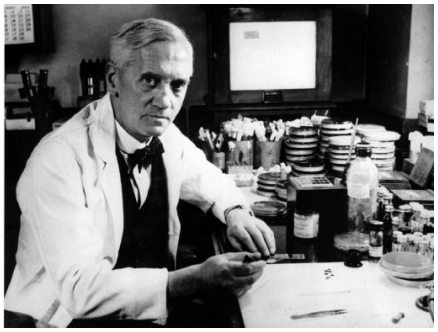
β-lactams access to peptidoglycan differs in Gram+ and Gram-



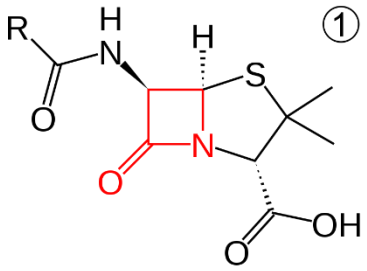




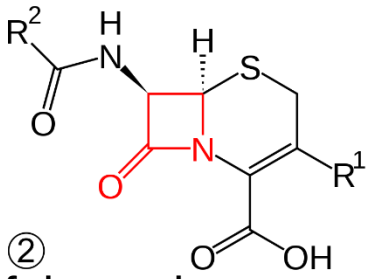
Penicillium



Fleming, Chain e Florey



① penicillina



② cefalosporina

Penicillina (1928)

Cefalosporina (1945)

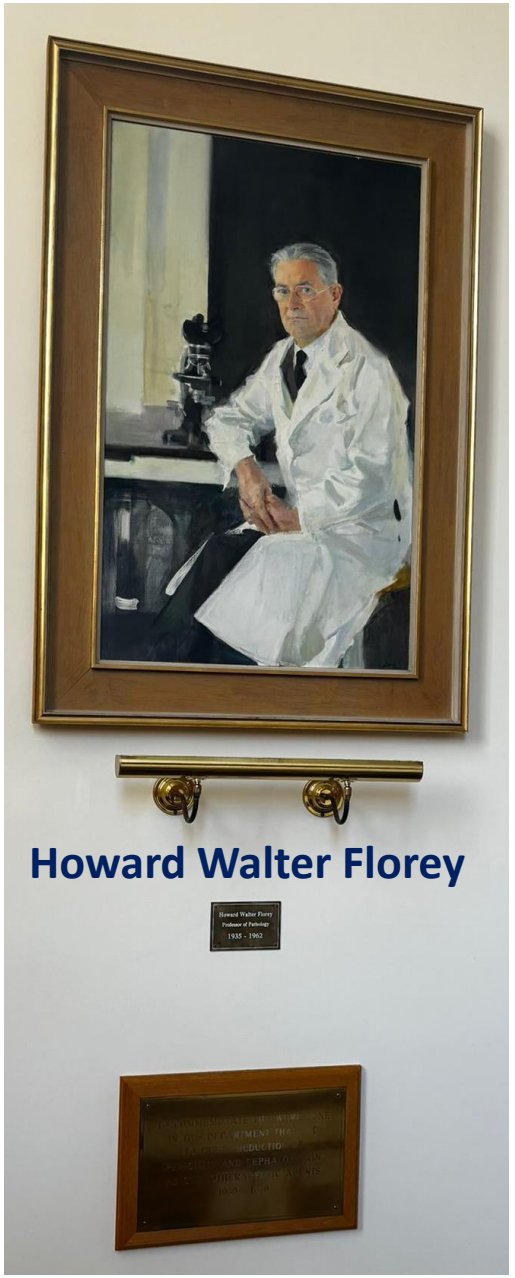
1945 1950 1955 1960 1965 1970 1975 1980 1985 1990 1995 2000 2005 2010 2015 2020 2025

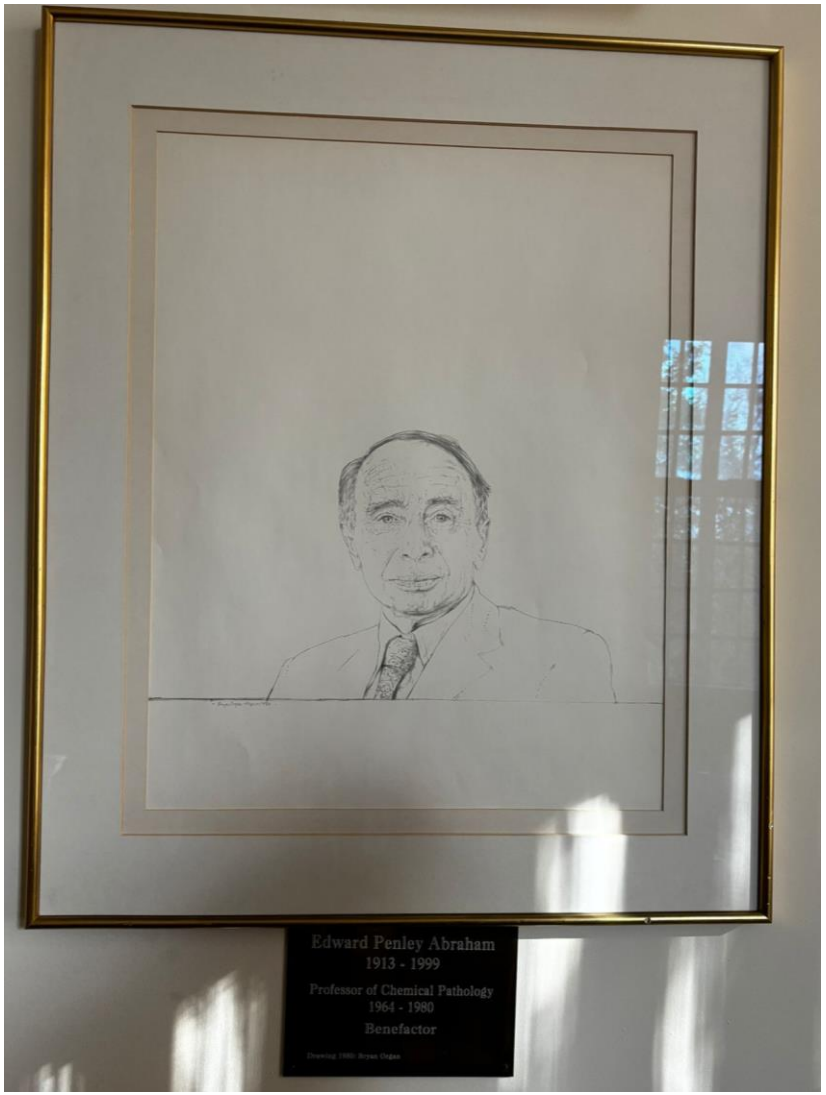
Acremonium, Cephalosporium

Giuseppe Brotzu

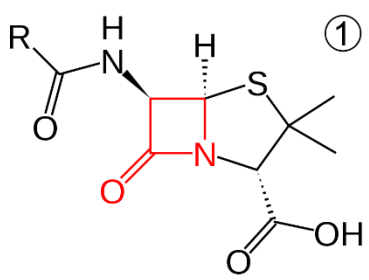
Edward Abraham


Ernst Boris Chain
Ernst Boris Chain was born June 19th, 1906 in Berlin, and graduated in chemistry from Friedrich Wilhelm University in 1930. He came to the UK in 1933 to escape Nazi persecution and began working at the Dunn School in 1935. With Howard Florey's concurrence, he followed up Fleming's abandoned work on penicillin. After the War, he worked at the Istituto Superiore di Sanita in Rome before returning to Imperial College as Head of the Biochemistry Department. He retired to Ireland and died on August 12th, 1979. Together with Fleming and Florey, he received the Nobel Prize for Physiology or Medicine in 1945.

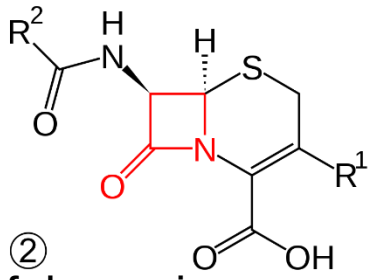

Howard Walter Florey
Howard Walter Florey
Professor of Pathology
1935 - 1962



Edward Penley Abraham
Benefactor



① penicillina

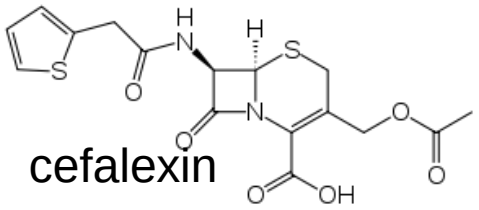


② cefalosporina

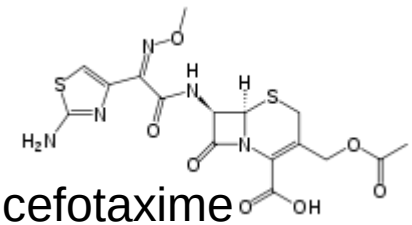
2a gen. cefalosporine

3° gen. cefalosporine

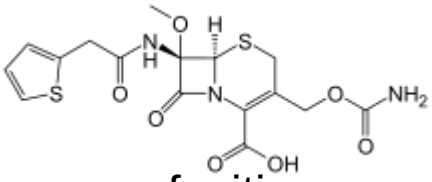
1945 1950 1955 1960 1965 1970 1975 1980 1985 1990 1995 2000 2005 2010 2015 2020 2025



cefalexin



cefotaxime



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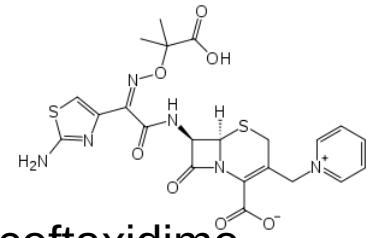
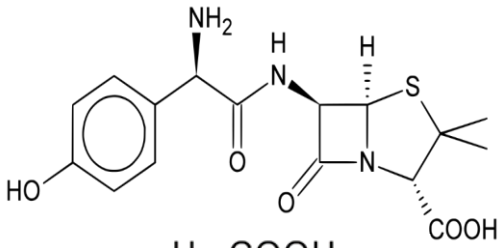
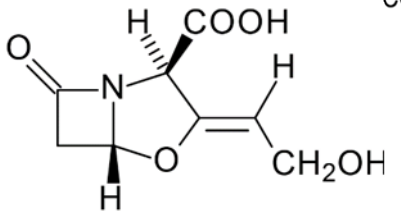


ceftaxidime

La strategia delle molecole suicide



amoxicillina



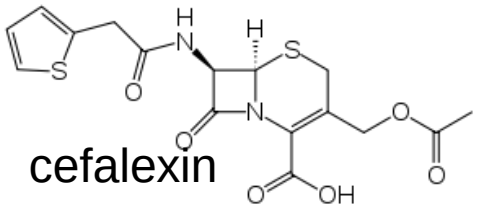
Acido clavulanico ***Streptomyces clavuligerus***

2a gen. cefalosporine

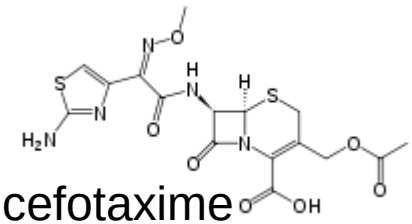
3° gen. cefalosporine

Amox, ac. clavulanico

1945 1950 1955 1960 1965 1970 1975 1980 1985 1990 1995 2000 2005 2010 2015 2020 2025



cefalexin



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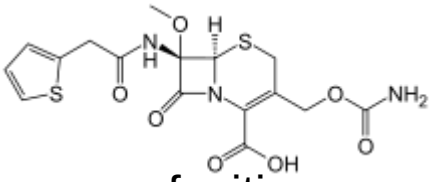

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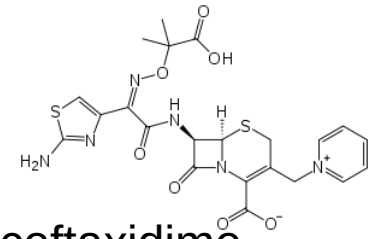

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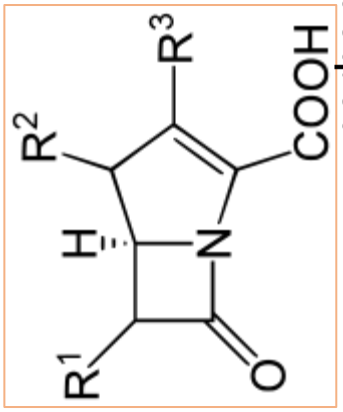
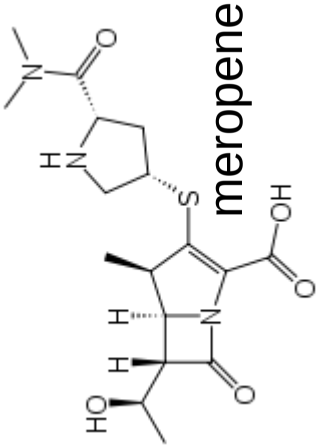
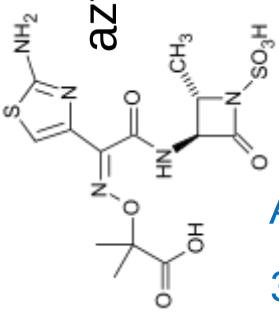


carbapenemi



meropenem



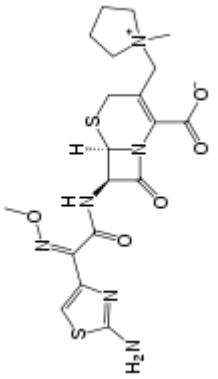
aztreonam

2025
2020
2015
2010
2005
2000
1995
1990
1985
1980
1975
1970
1965
1960
1955
1950
1945

ceftazidime/avibactam

meropenem/varborbactam

5a gen. cefalosp



Cefepime

meropenem

4a gen. cefalosporine

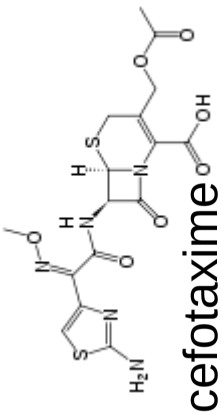
imipenem

aztreonam

Amox, ac. clavulanico

3° gen. cefalosporine

2a gen. cefalosporine



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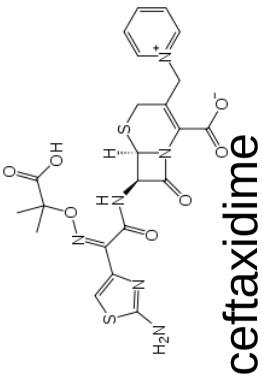

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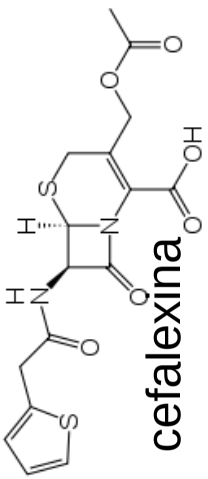
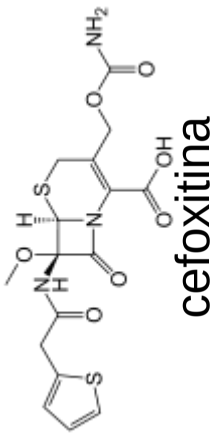


cefalexina



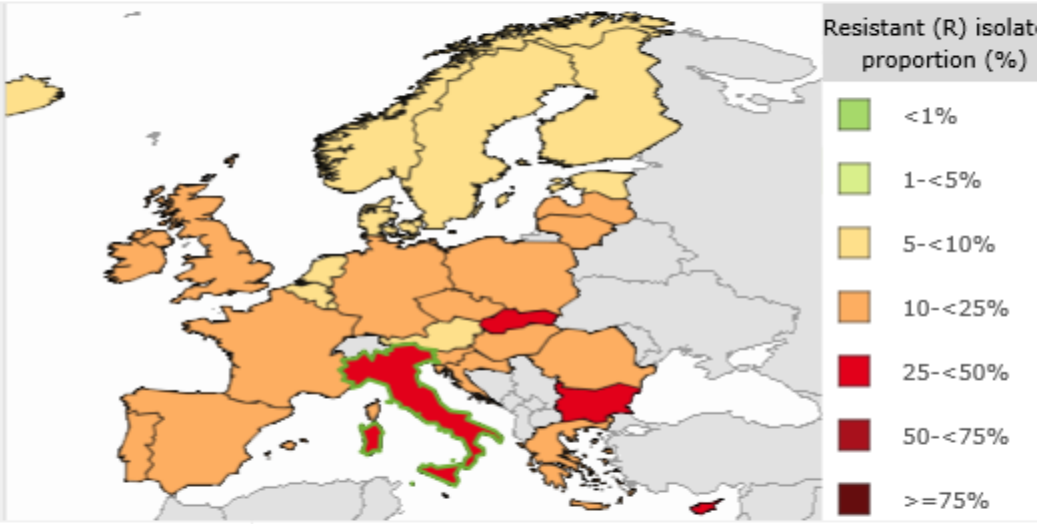
cefoxitina

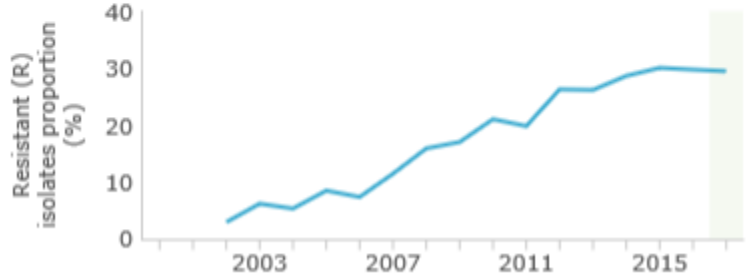
Surveillance Atlas of Infectious Diseases

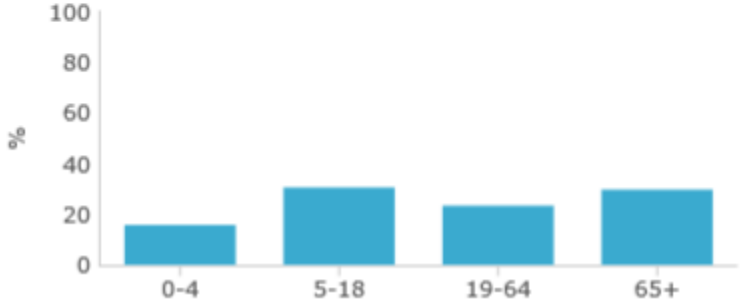
Antimicrobial resistance | **Escherichia coli** | **Third-generation cephalosporins**

Resistant (R) isolates proportion | 2017

Estonia	8.8
Finland	6.9
France	10.2
Germany	12.3
Greece	18.3
Hungary	20.1
Iceland	6.1
Ireland	12.0
Italy	29.5
Latvia	22.0
Lithuania	16.8







Surveillance Atlas of Infectious Diseases

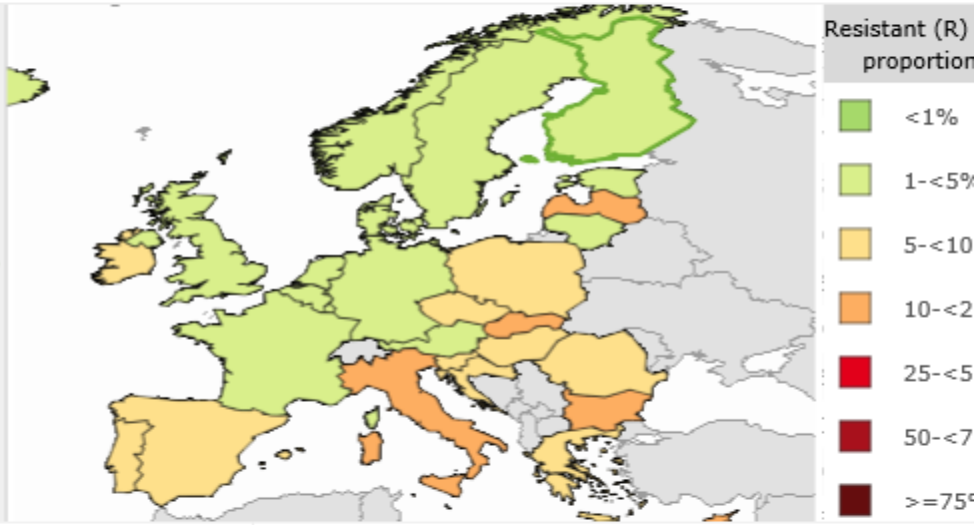
Antimicrobial resistance

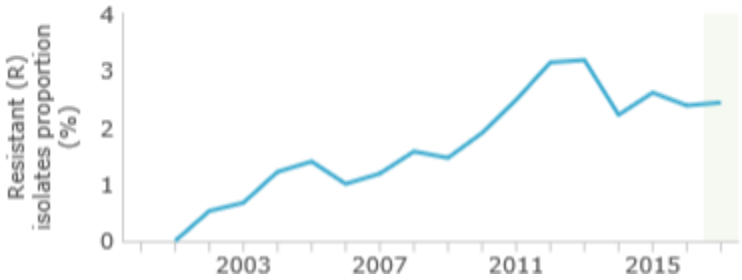
Escherichia coli

Combined resistance (third-generation cephalosporin, fluoroquinolones and aminoglycoside)

Resistant (R) isolates proportion 2017

Finland	2.4
France	3.0
Germany	3.7
Greece	9.8
Hungary	8.2
Iceland	1.5
Ireland	5.7
Italy	13.7
Latvia	11.2
Lithuania	4.4
Luxembourg	3.5





Surveillance Atlas of Infectious Diseases

Antimicrobial resistance | **Escherichia coli** | **Carbapenems** | **Resistant (R) isolates proportion**

2017

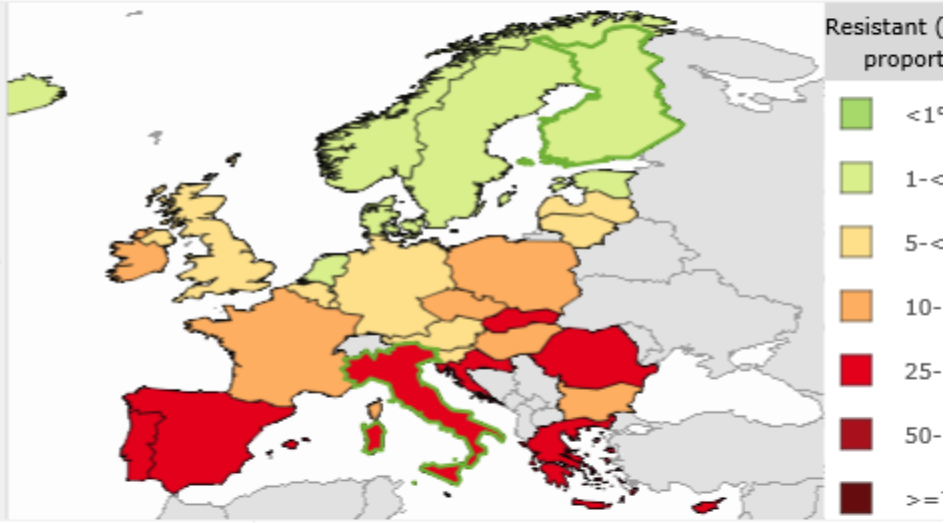
Region	Resistant (R) isolates proportion (%)
Austria	0.0
Belgium	0.0
Bulgaria	0.0
Croatia	0.0
Cyprus	1.3
Czech Republic	0.0
Denmark	0.0
Estonia	0.0
Finland	0.0

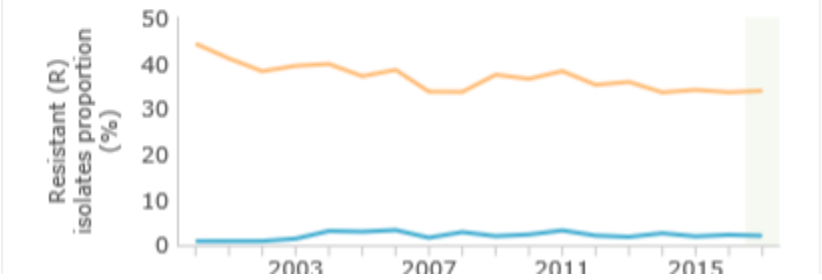
Surveillance Atlas of Infectious Diseases

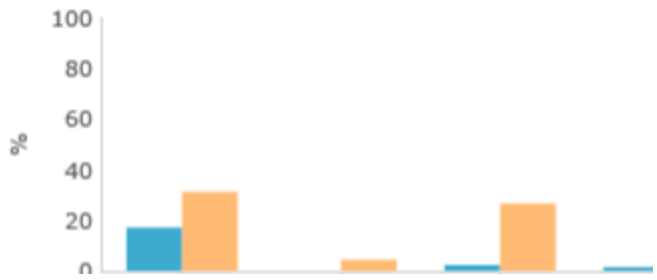
Antimicrobial resistance | **Staphylococcus aureus** | **Meticillin (MRSA)**

Resistant (R) isolates proportion | 2017

Iceland	1.4
Ireland	16.3
Italy	33.9
Latvia	5.7
Lithuania	8.8
Luxembourg	9.5
Malta	42.1
Netherlands	1.5
Norway	1.0
Poland	15.2
Portugal	39.2





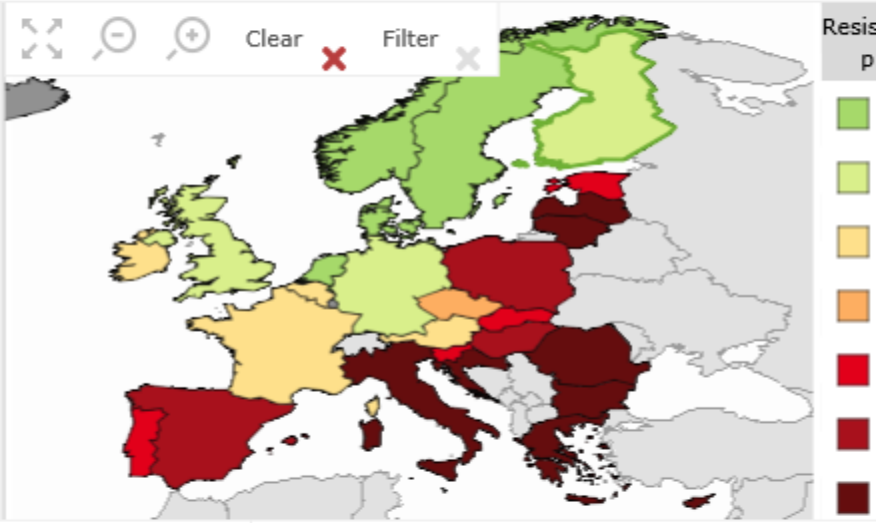


Surveillance Atlas of Infectious Diseases

Antimicrobial resistance | **Acinetobacter spp.** | **Carbapenems**

Resistant (R) isolates proportion | **2017**

Estonia	33.3
Finland	2.7
France	6.2
Germany	4.4
Greece	94.8
Hungary	52.5
Iceland	-
Ireland	6.3
Italy	78.7
Latvia	79.4
Lithuania	88.5

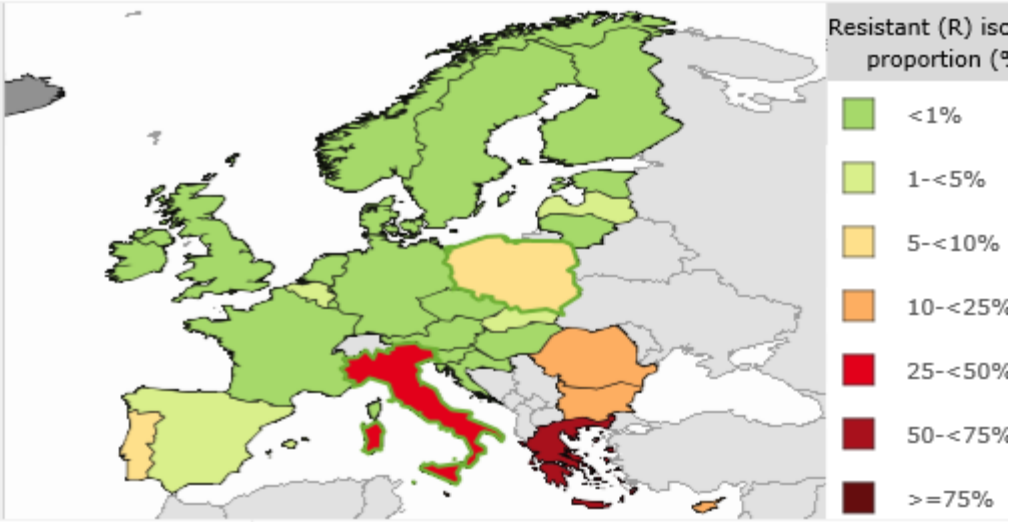


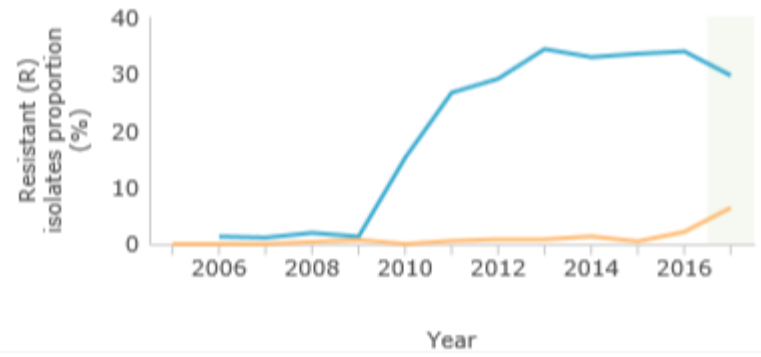
Surveillance Atlas of Infectious Diseases

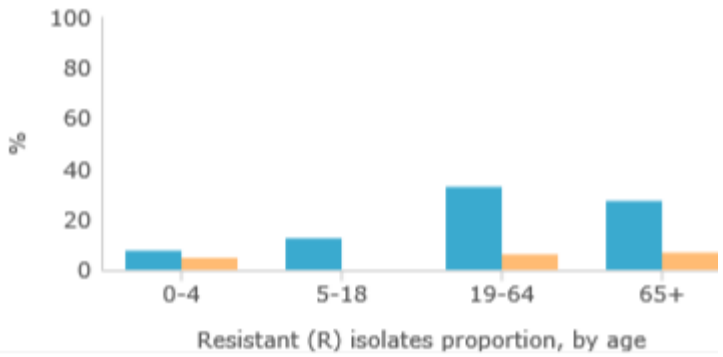
Antimicrobial resistance | **Klebsiella pneumoniae** | **Carbapenems**

Resistant (R) isolates proportion ◀ ◀◀ **2017** ▶▶

Region	Resistant (R) isolates proportion (%)
Austria	1.0
Belgium	1.1
Bulgaria	12.4
Croatia	0.0
Cyprus	15.5
Czech Republic	0.4
Denmark	0.3
Estonia	0.0
Finland	0.3



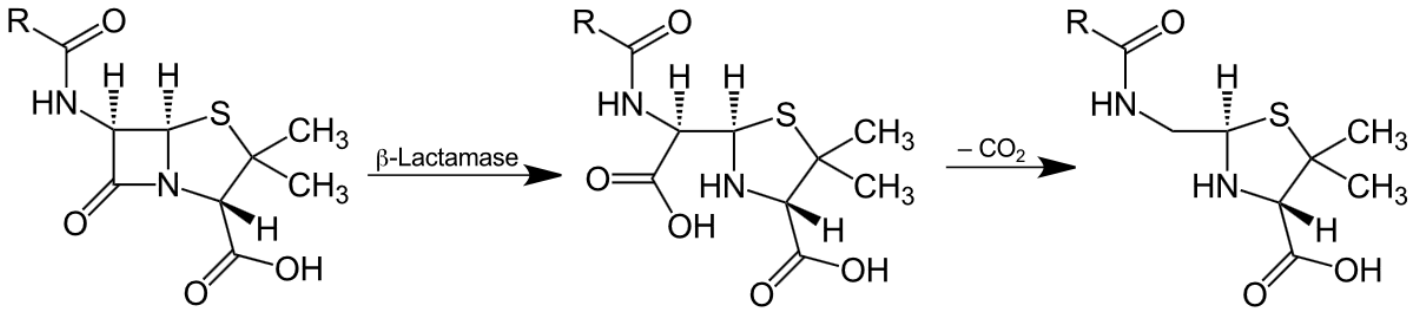




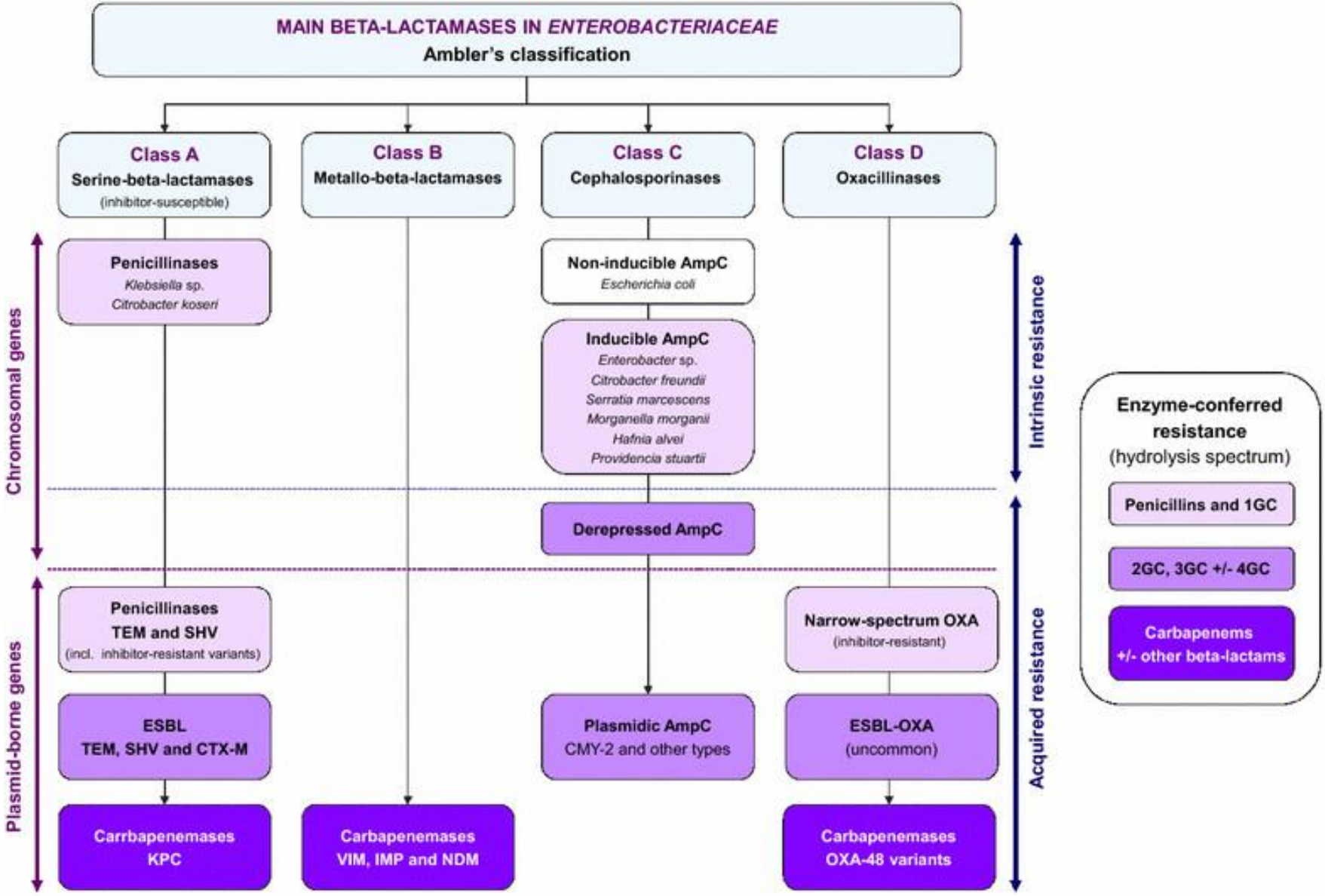
Italy Poland

Beta-lactams: resistance mechanisms

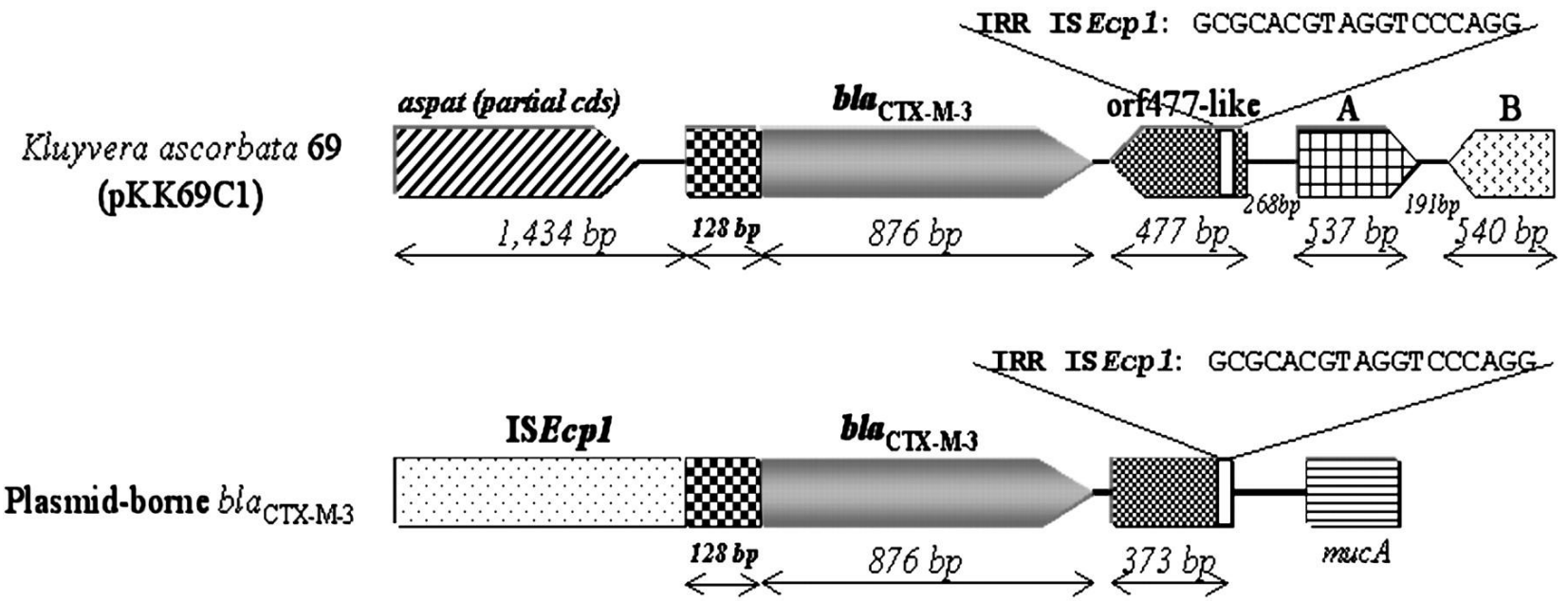
beta-lactamases



La prima penicillinasi fu descritta da Abraham and Chain in 1940 in *Escherichia coli* prima che la penicillina fosse messa in commercio per uso clinico.
Beta-lattamici resistenti alle penicillinasi vennero sviluppate come la meticillina ma presto anche per queste molecule vennero descritti ceppi resistenti

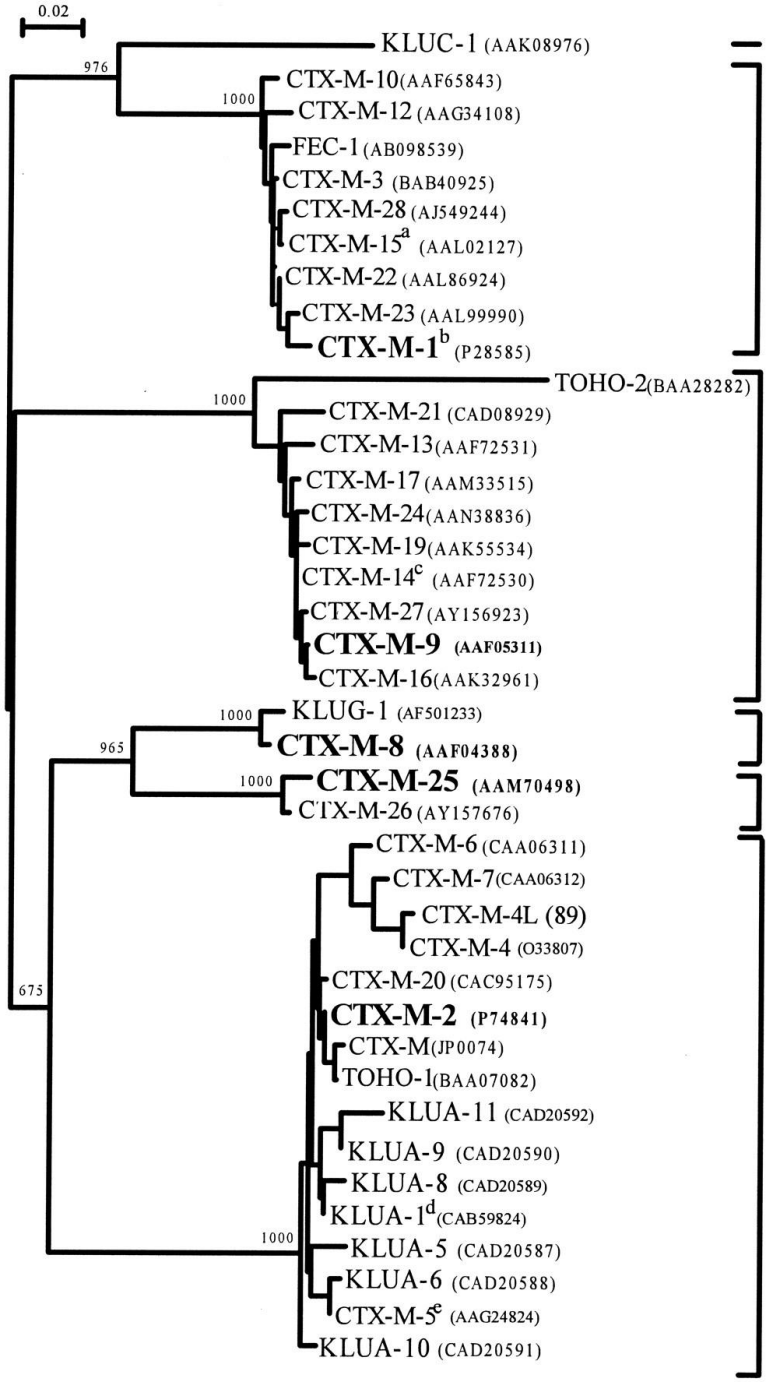

MAIN BETA-LACTAMASES IN ENTEROBACTERIACEAE
Ambler's classification
Class A
Serine-beta-lactamases
(inhibitor-susceptible)
Class B
Metallo-beta-lactamases
Class C
Cephalosporinases
Class D
Oxacillinases
Penicillinases
Klebsiella sp.
Citrobacter koseri
Non-inducible AmpC
Escherichia coli
Inducible AmpC
Enterobacter sp.
Citrobacter freundii
Serratia marcescens
Morganella morganii
Hafnia alvei
Providencia stuartii
Derepressed AmpC
Penicillinases
TEM and SHV
(incl. inhibitor-resistant variants)
ESBL
TEM, SHV and CTX-M
Carrbapenemases
KPC
Carbapenemases
VIM, IMP and NDM
Plasmidic AmpC
CMY-2 and other types
Narrow-spectrum OXA
(inhibitor-resistant)
ESBL-OXA
(uncommon)
Carbapenemases
OXA-48 variants
Chromosomal genes
Plasmid-borne genes
Intrinsic resistance
Acquired resistance
Enzyme-conferred resistance
(hydrolysis spectrum)
Penicillins and 1GC
2GC, 3GC +/- 4GC
Carbapenems
+/- other beta-lactams

Kluyvera ascorbata is the progenitor of $bla_{CTX\text{-}M\text{-}3}$



Rodríguez M M et al. Antimicrob. Agents Chemother. 2004;48:4895-4897

Antimicrobial Agents and Chemotherapy

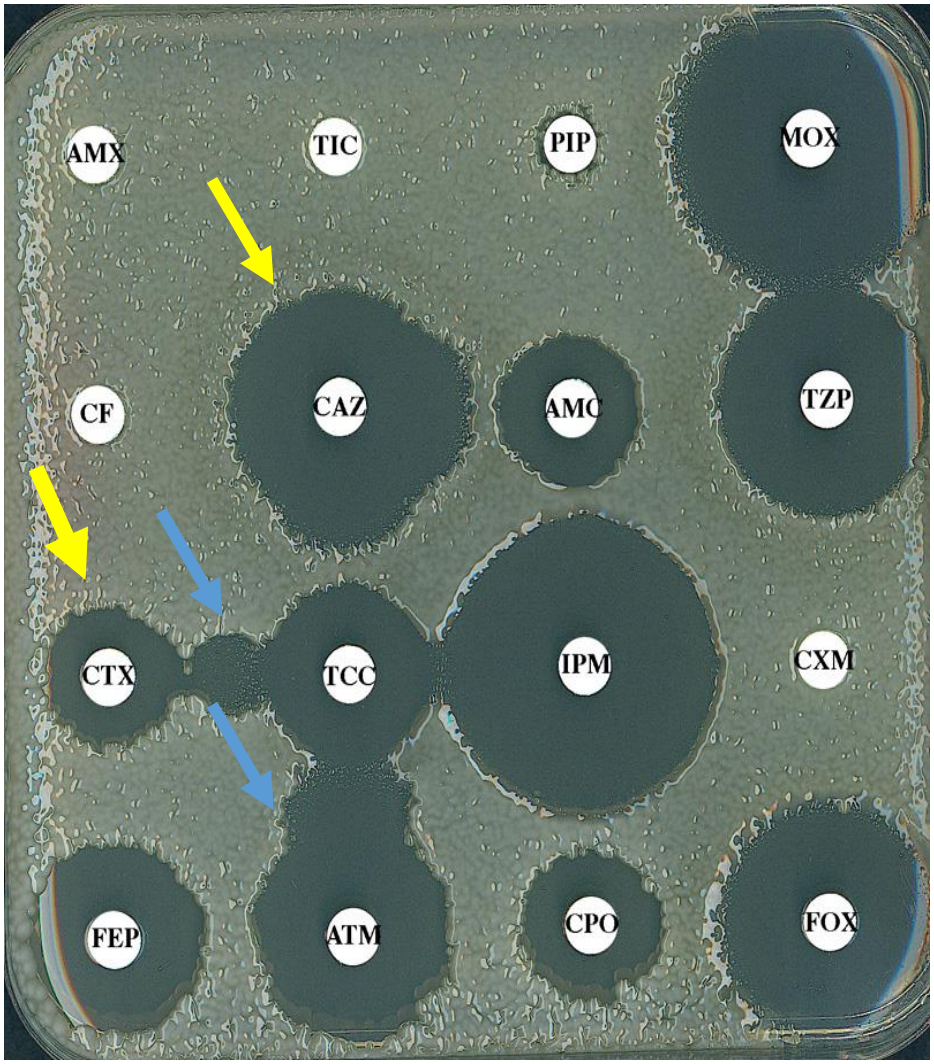


Kluyvera ascorbata

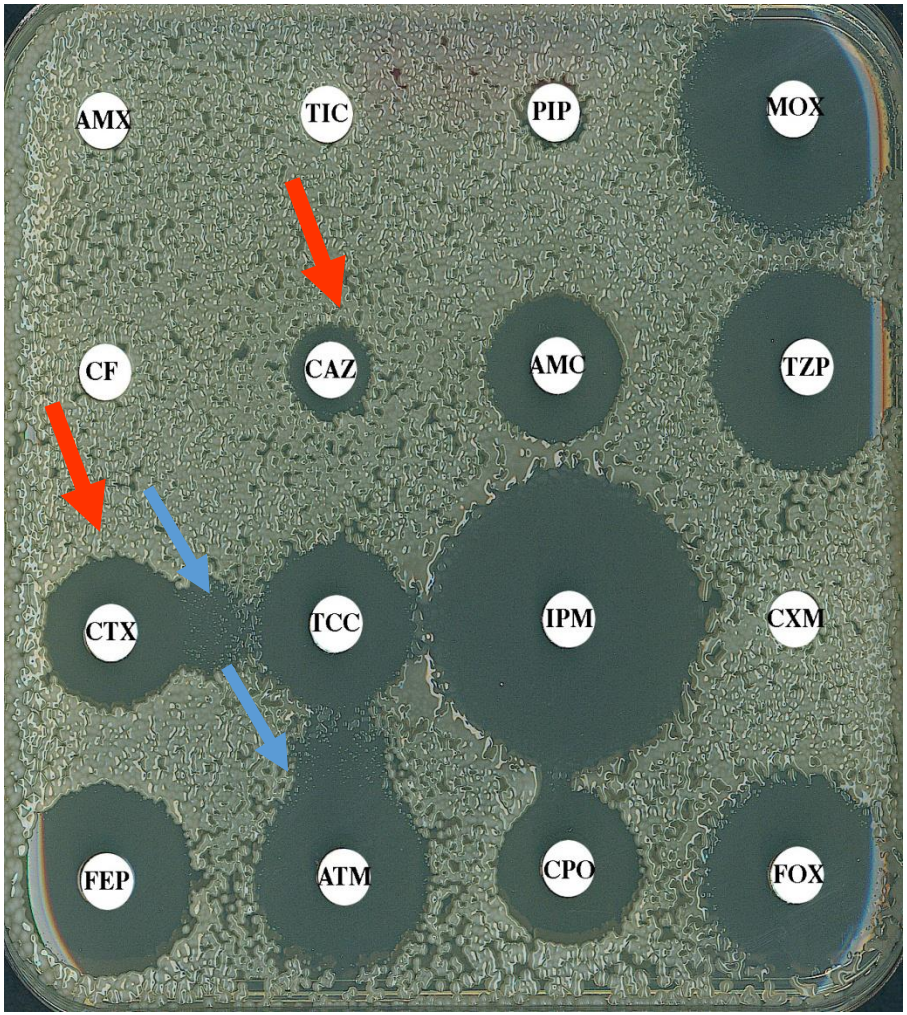
Kluyvera georgiana

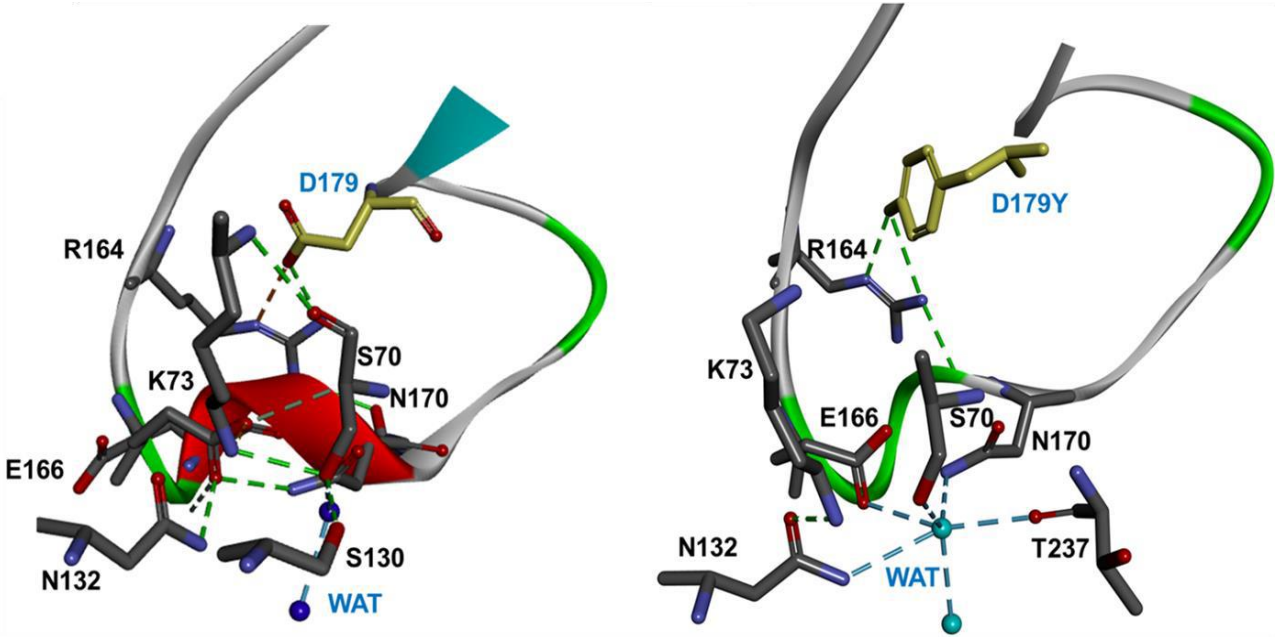
Kluyvera ascorbata

CTX-M-3



CTX-M-15





KPC-3

KPC-31

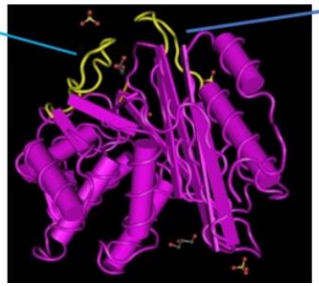
Replacing aspartic acid in tyrosine at position 179 changes the shape of the Ω-Loop. The residue K73 maintains the ionic bond with E166 and moves away from S70. Catalytic water is better positioned between E166-N170-S70 in the D179Y variant than KPC-3 against CZA. This position prevents the deacilation of MEM and promotes the hydrolysis of CAZ. In addition, an increase in IC50 is observed for AVI inhibition of CAZ hydrolysis in variant D179Y

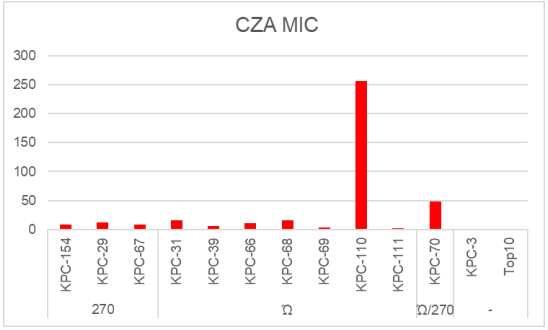
(Taracila MA, Different Conformations Revealed by NMR Underlie Resistance to Ceftazidime/Avibactam and Susceptibility to Meropenem and Imipenem among D179Y Variants of KPC β-Lactamase. 2022)

```
KPC-67   KLEQDFGGSI--//--FRLDRW----ELELNSAIPGDARDTSS---PRAVTESL~PTGRAPIVLAVYTRAPNKDDKDDKDD--------KYSEAVIAA
KPC-66   KLEQDFGGSI--//--FRLDRW----EL----NSAIPGDARDTSS---PRAVTESL~PTGRAPIVLAVYTRAPNKDD----------KYSEAVIAA
KPC-68   KLEQDFGGSI--//--FRLDRW----ELELNSAIPGDARDTSSSSPRAVTESL~PTGRAPIVLAVYTRAPNKDD----------KYSEAVIAA
KPC-69   KLEQDFGGSI--//--FRLDRWGLELELNSAIPGDARDTSS---PRAVTESL~PTGRAPIVLAVYTRAPNKDD----------KYSEAVIAA
KPC-70   KLEQDFGGSI--//--FRLDRW----ELELNSAIPGDARYTSS---PRAVTESL~PTGRAPIVLAVYARAPNKDD----------KYSEAVIAA
KPC-29   KLEQDFGGSI--//--FRLDRW----ELELNSAIPGDARDTSS---PRAVTESL~PTGRAPIVLAVYTRAPNKDDKDD--------KYSEAVIAA
KPC-31   KLEQDFGGSI--//--FRLDRW----ELELNSAIPGDARYTSS---PRAVTESL~PTGRAPIVLAVYTRAPNKDD----------KYSEAVIAA
KPC-49   KLEQDFGGSI--//--FRLDSW----ELELNSAIPGDARDTSS---PRAVTESL~PTGRAPIVLAVYTRAPNKDD----------KYSEAVIAA
KPC-39   KLEQDFGGSI--//--FRLDRW----ELELNSTIPGDARDTSS---PRAVTESL~PTGRAPIVLAVYTRAPNKDD----------KYSEAVIAA
KPC-3    KLEQDFGGSI--//--FRLDRW----ELELNSAIPGDARDTSS---PRAVTESL~PTGRAPIVLAVYTRAPNKDD----------KYSEAVIAA
KPC-110  KLEQDFGRSI--//--FRLDRW----ELELNSAIPGDARYTSS---PRAVTESL~PTGRAPIVLAVYTRAPNKDD----------KYSEAVIAA
KPC-154  KLEQDFGGSI--//--FRLDRW----ELELNSAIPGDARDTSS---PRAVTESL~PTGRAPIVLAVYTRAPNKDDKYSRAPNKDDKYSEAVIAA
KPC-111  KLEQDFGGSI--//--FRLDRW---ELELNSAILGDARYTSS---PRAVTESL~PTGRAPIVLAVYTRAPNKDD----------KYSEAVIAA
```

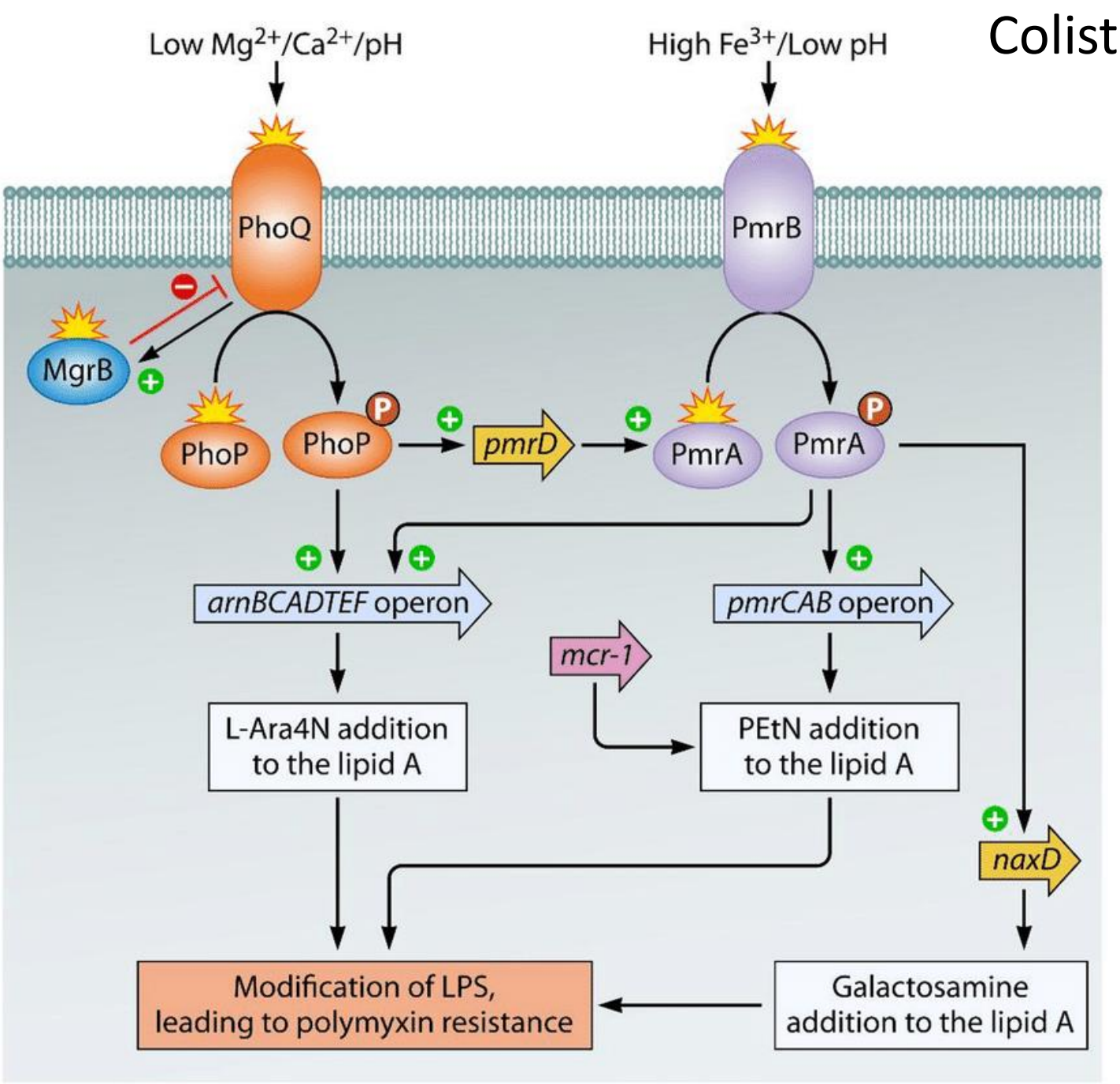
Ω-LOOP

270-LOOP





Colistin resistance



Fosfomycine resistance

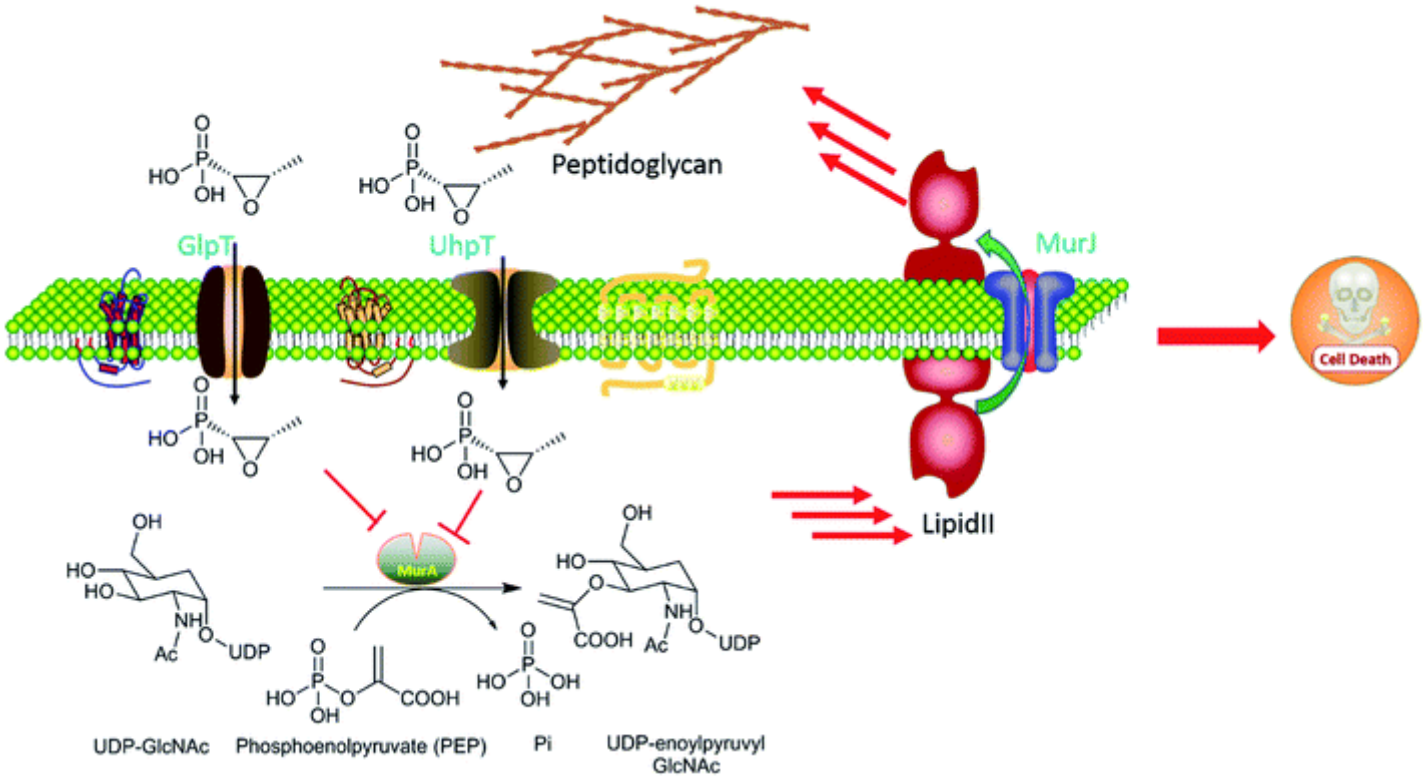
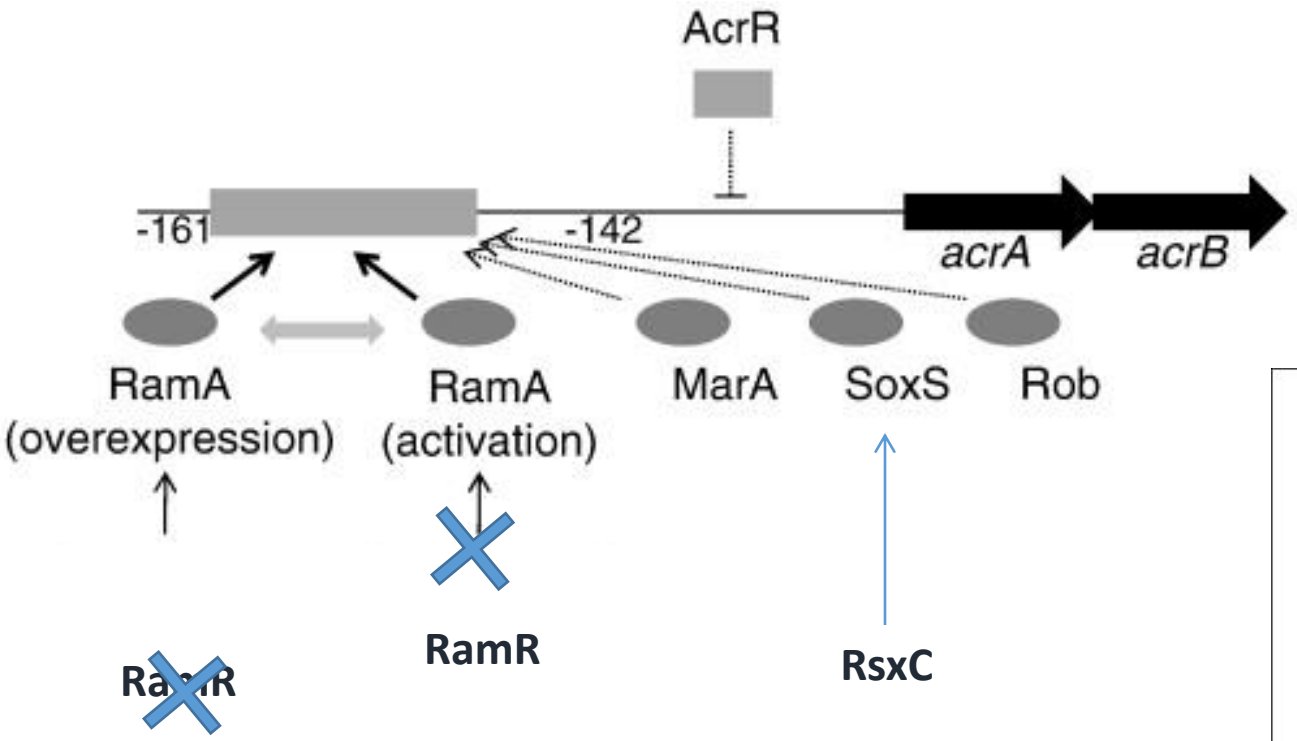
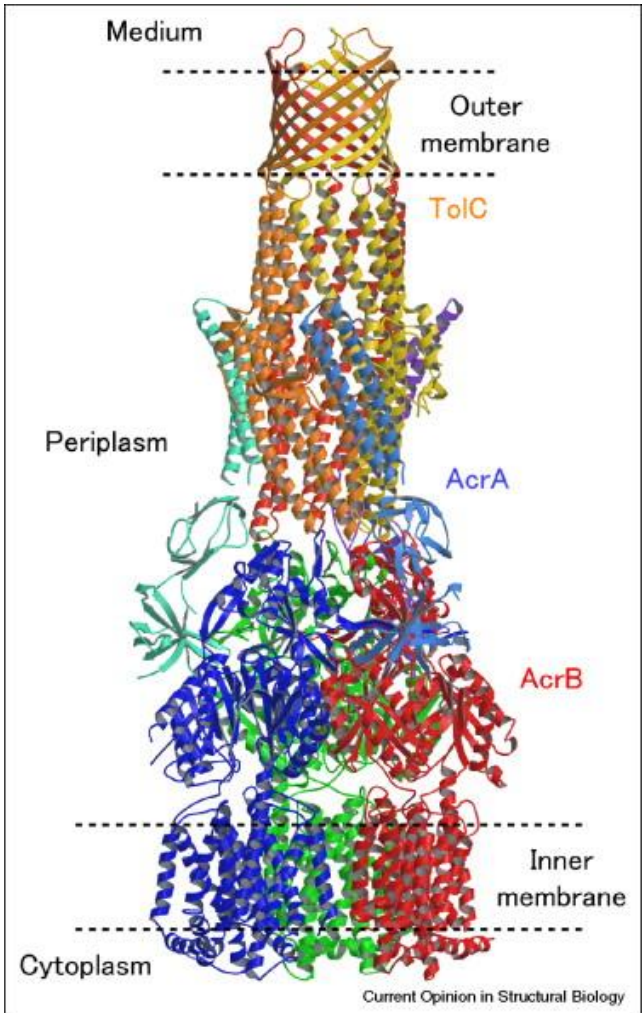


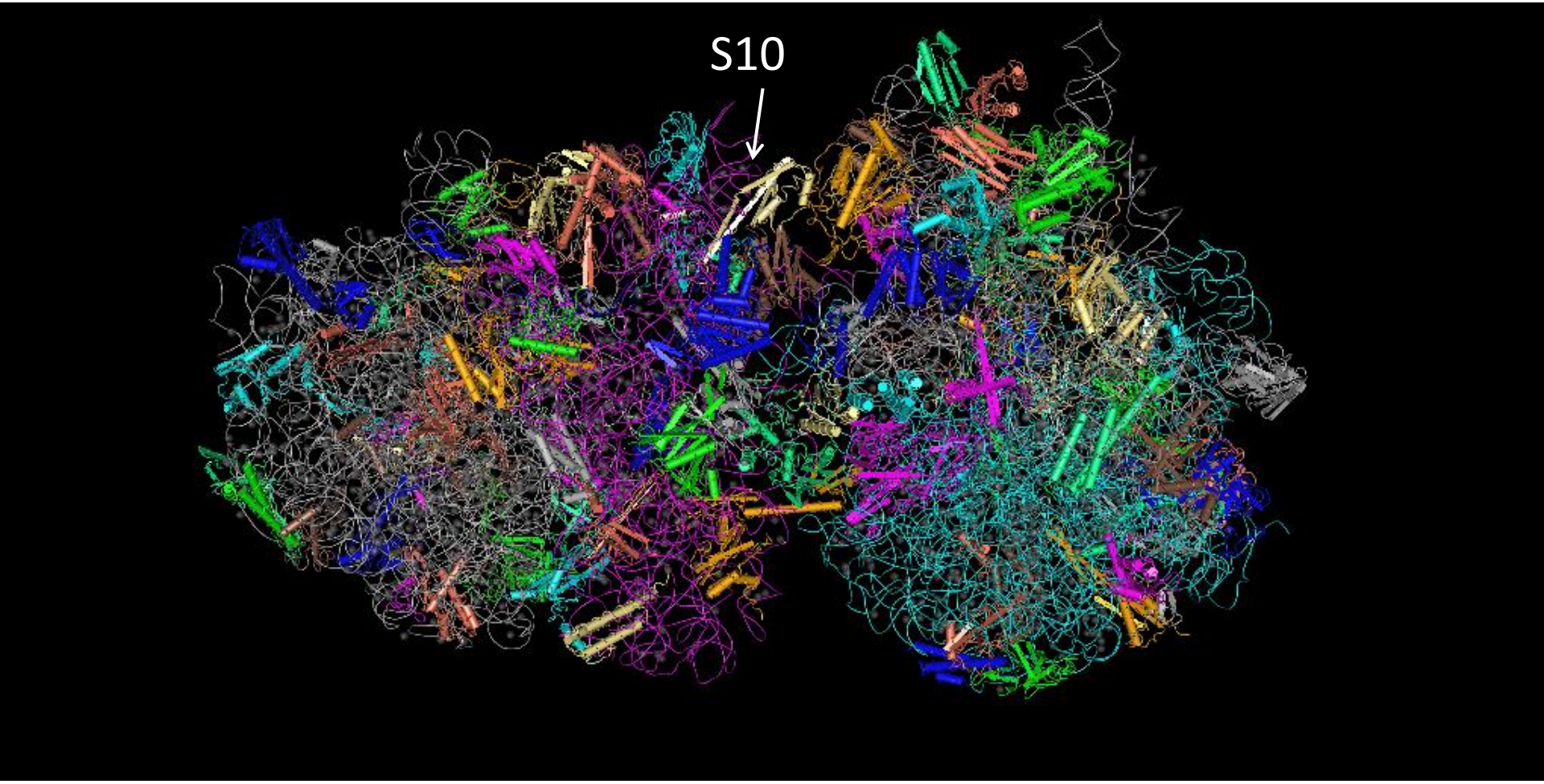
Fig. 2 Mechanism of action of fosfomycin. Chemical structure of fosfomycin mimics both glycerol-3-P (G3P) and glucose-6-P (G6P), which are transported by transporters GlpT and UhpT, respectively. MurA catalyzes the formation of UDP-GlcNac-3-O-enolpyruvate, a peptidoglycan precursor, from UDP-GlcNAc and PEP during the first step of peptidoglycan biosynthesis. Once fosfomycin (F) is present, it is transported inside the cell by GlpT and UhpT, blocking the UDP-GlcNac-3-O-enolpyruvate synthesis by mimicking the original substrate of MurA, PEP, avoiding cell wall synthesis and leading to cell death.



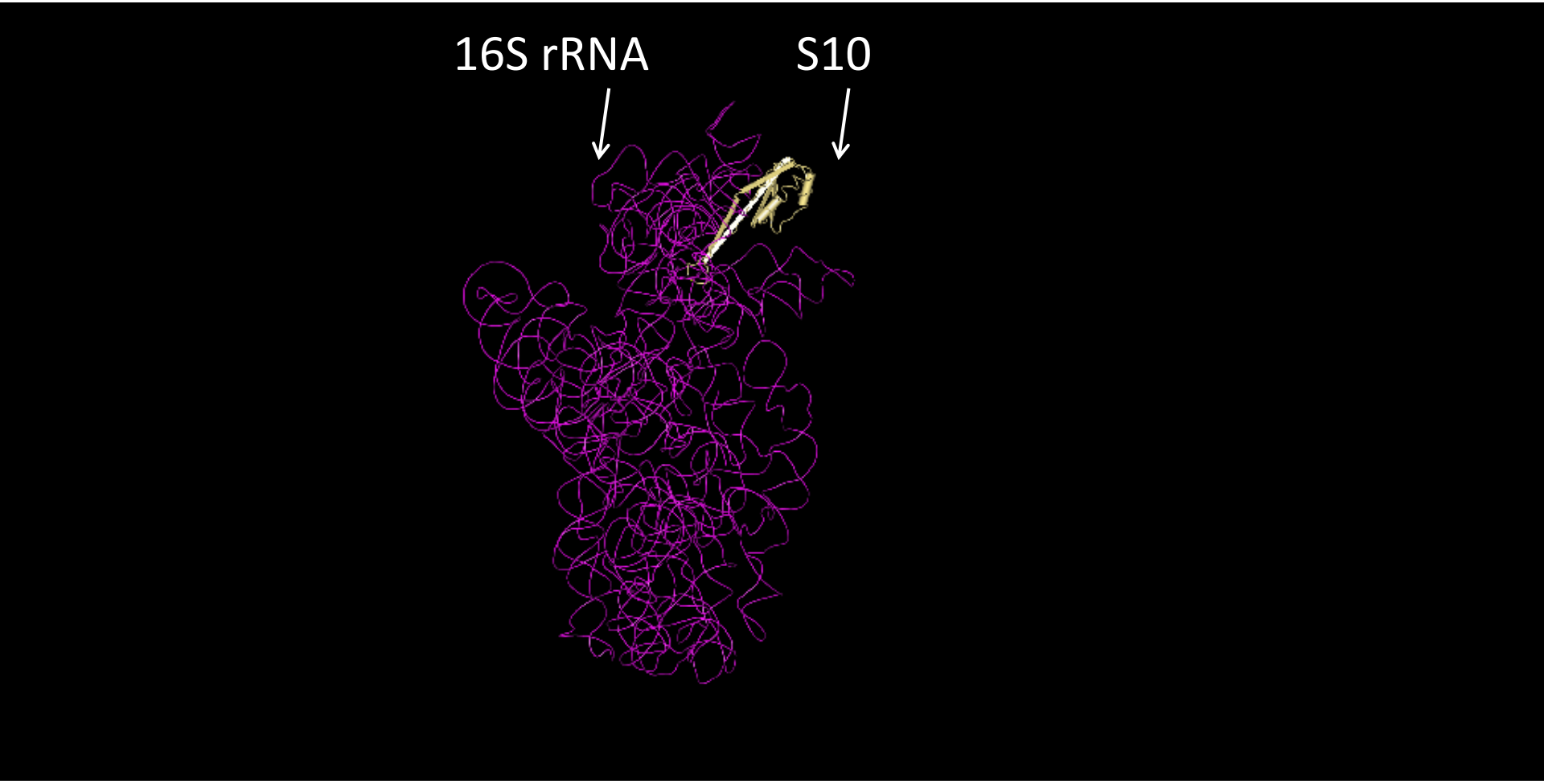


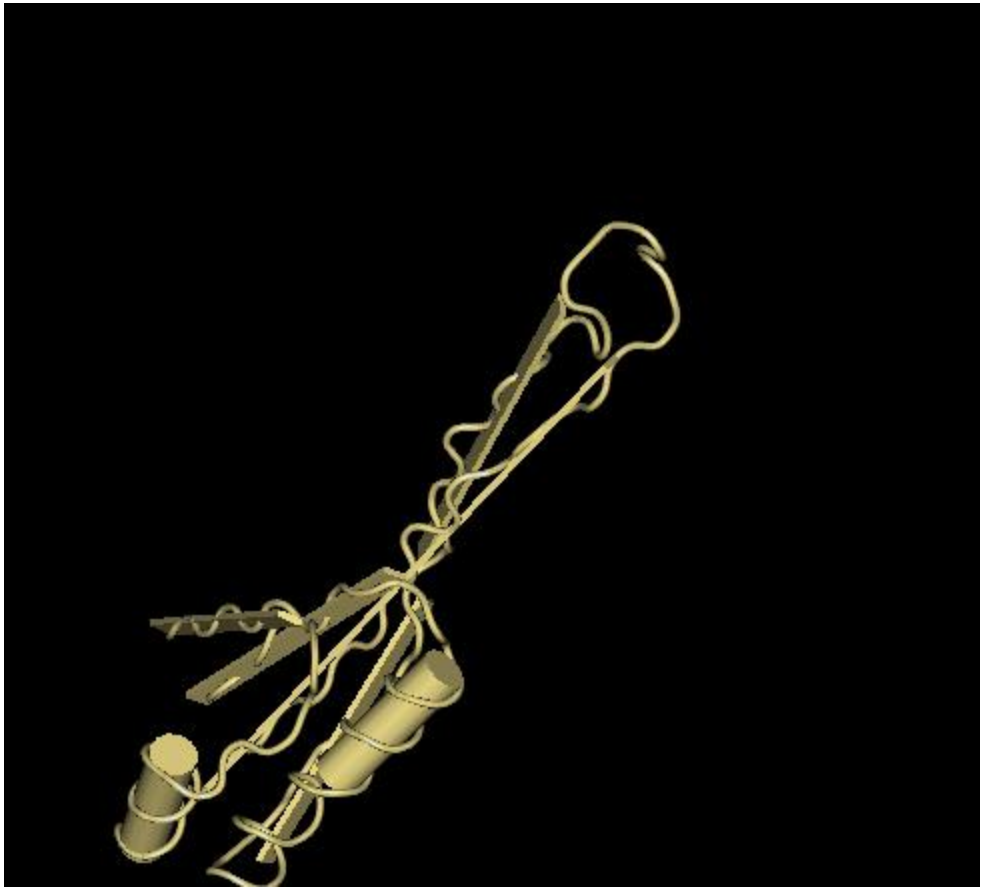
Tigecycline resistance

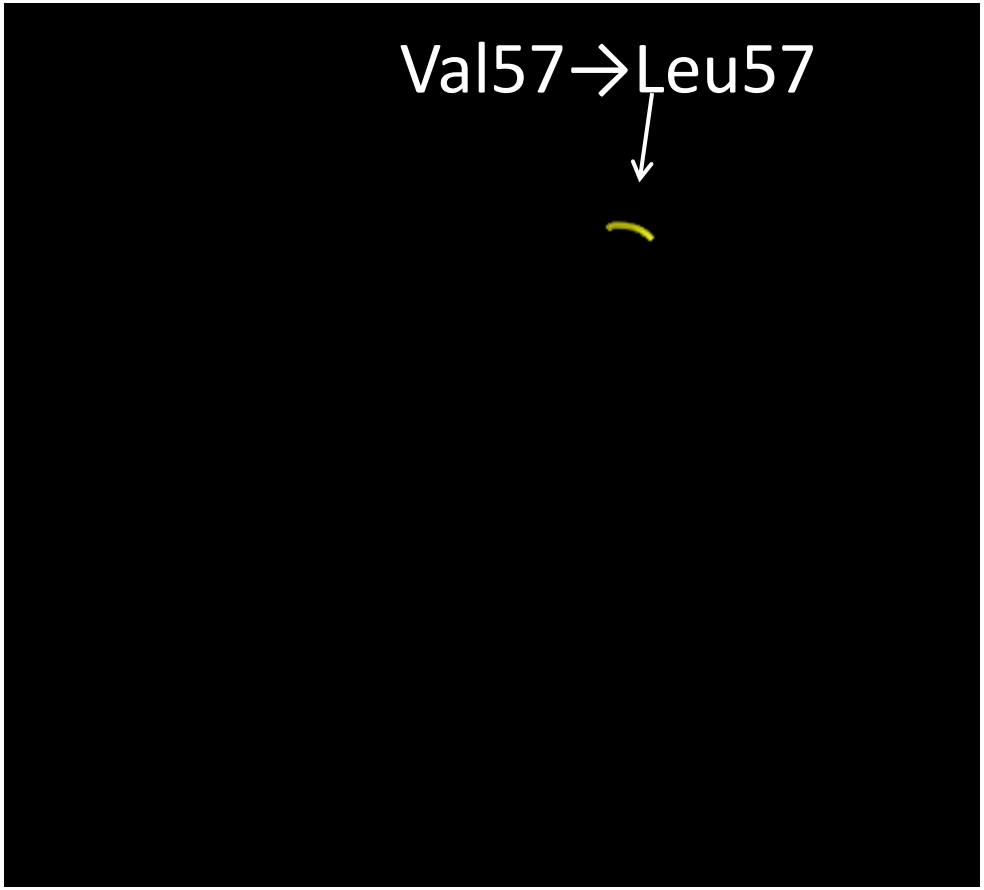
Il ceppo resistente KP4-R non ha mutazioni nelle pompe ad efflusso ma solo una mutazione Val57→Leu 57 nella proteina S10 della subunità 30S del ribosoma.
E' questo il meccanismo di resistenza?

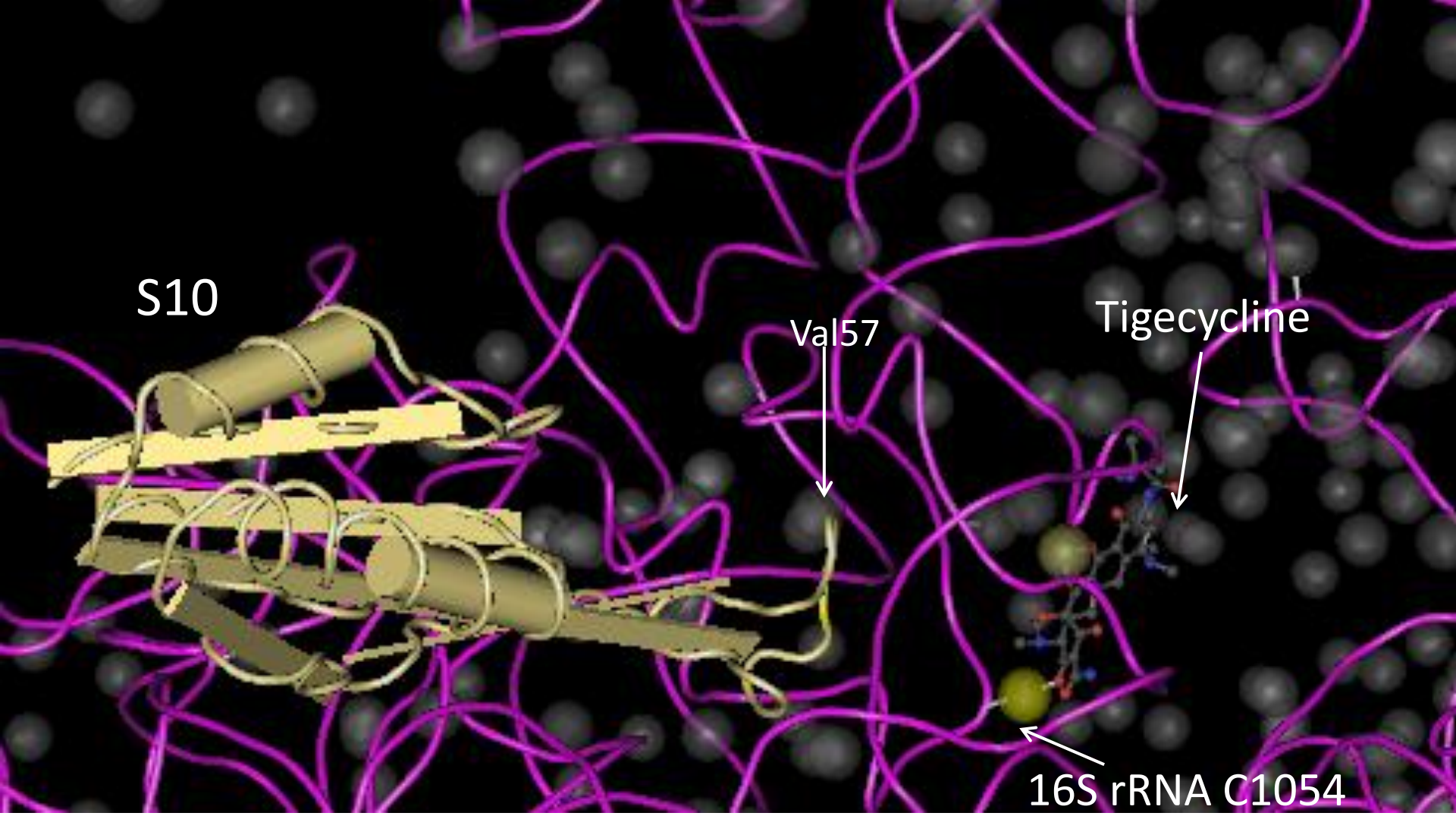


3D model of the 30S Ribosome of *Thermus Thermophilus* with Tigecycline.
(mmdb_4G5T; Jenner et al., PNAS 2013)

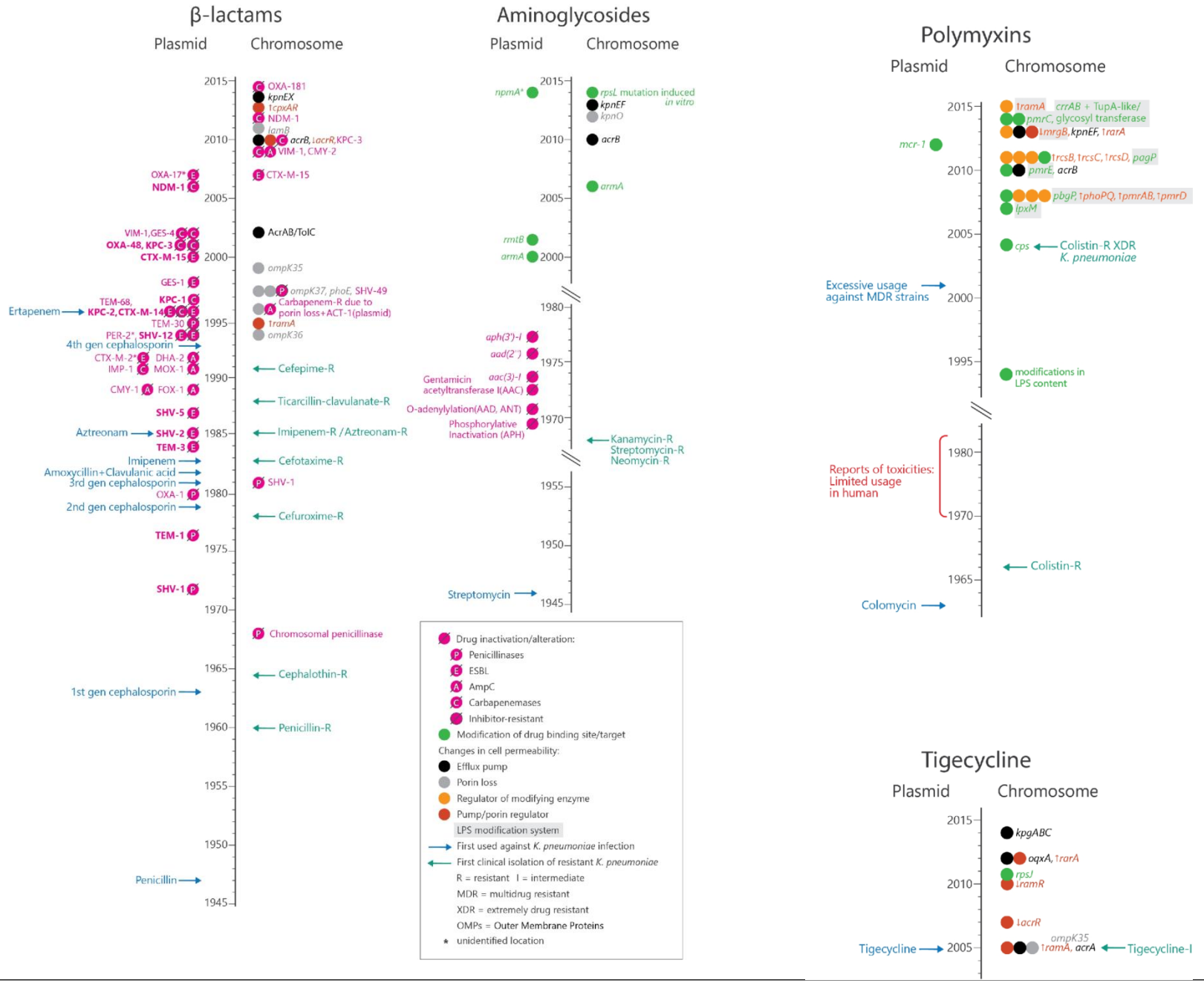

16S rRNA
S10




Val57→Leu57



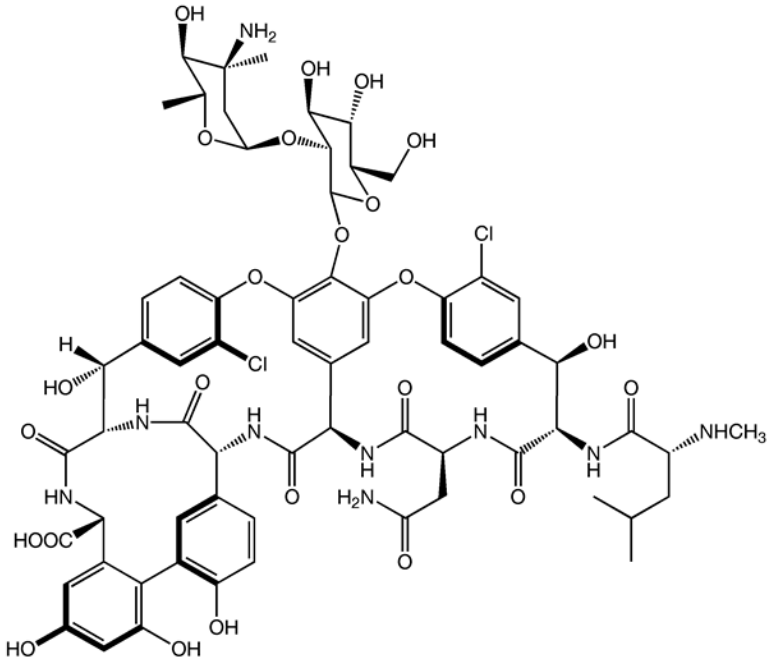
Tigecycline linked to nucleotide C1054 of 16S rRNA (pink ribbon) via a coordinated Mg2+ ion (yellow ball). The Val57 codon of S10 is located at approx. 8 Å from this link.
Tigecycline coordinates a second Mg2+ ion that facilitates an indirect interaction with h31 of 16S rRNA.
(Garcia-Fernandez et al., 2013)



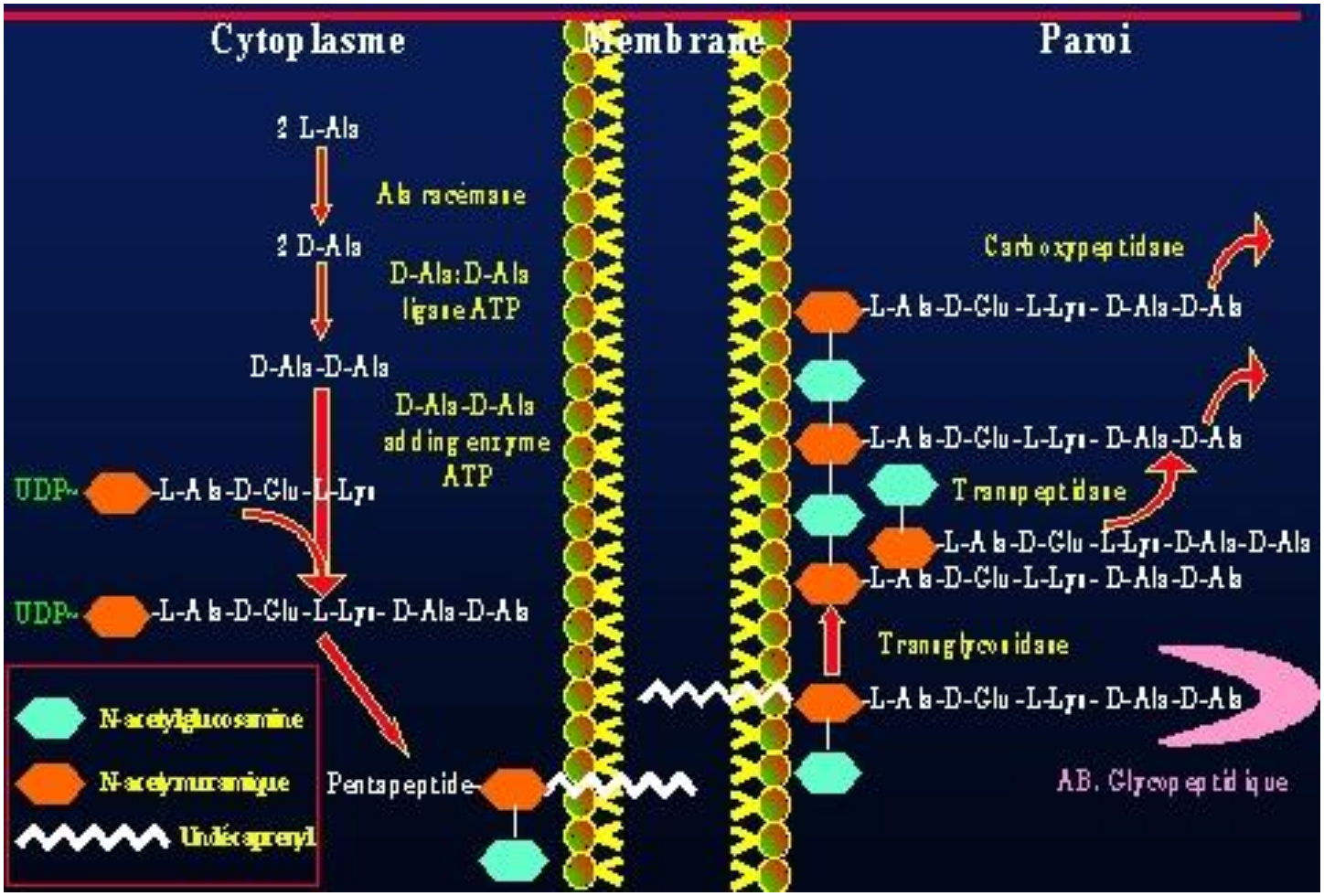
From:Shiri Navon-Venezia Kira Kondratyeva Alessandra Carattoli

Klebsiella pneumoniae: a major worldwide source and shuttle for antibiotic resistance

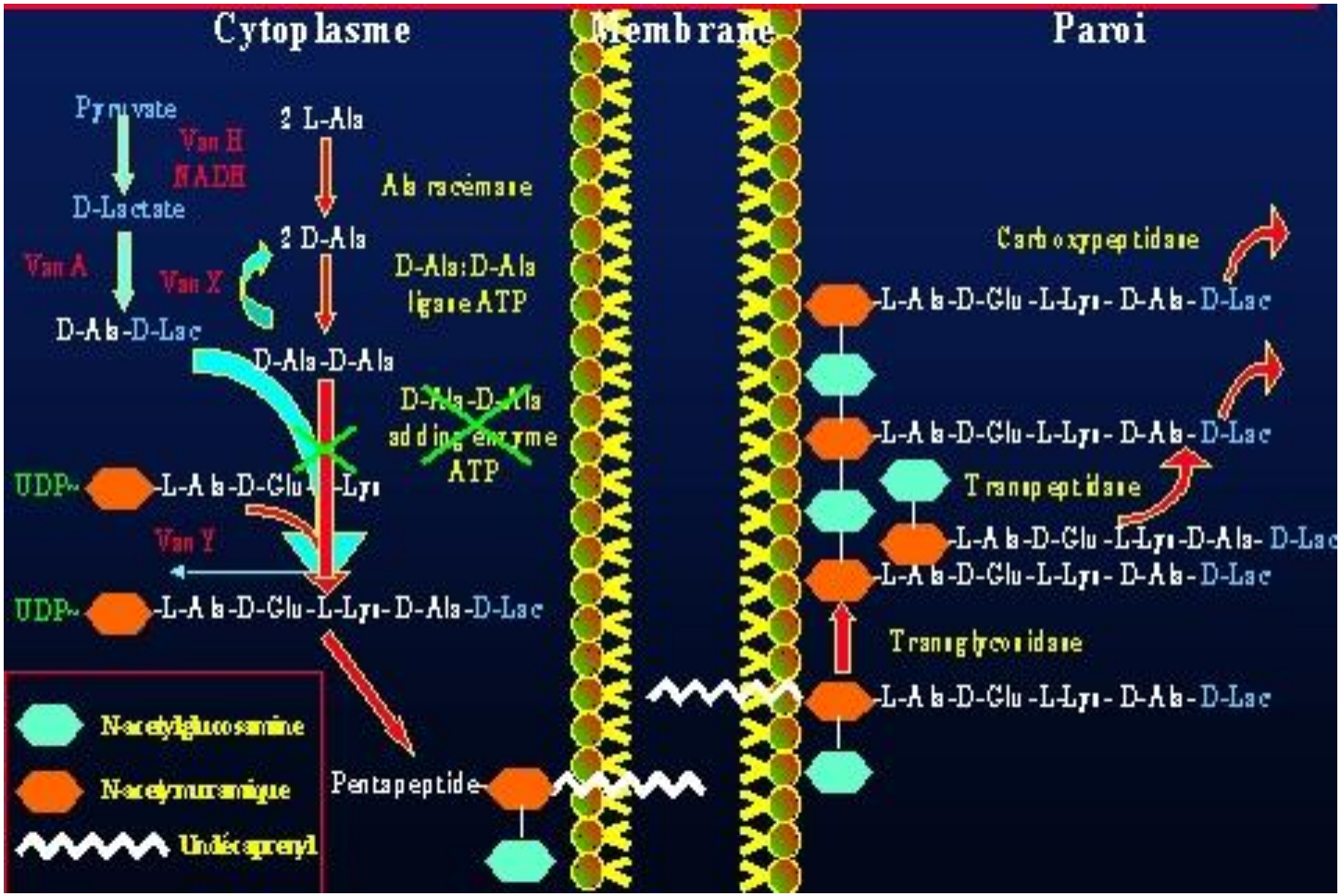
vancomicina



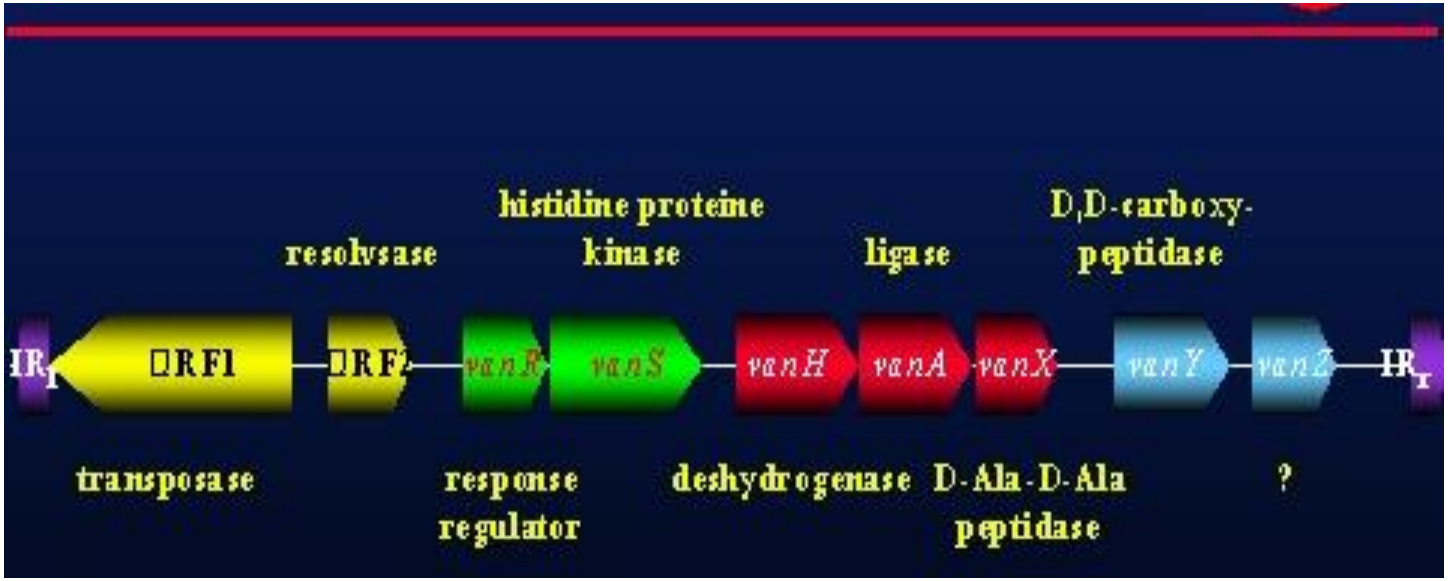
Meccanismo d'azione della vancomicina



Resistenza alla vancomicina



Transposon Tn1546



10851 bp
9 proteins