Introduction to antibiotics

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What is the role of the micro lab?

1) Identify if a pathogen might be causing infection
2) If a pathogen might be causing infection, then determine the pathogen's identity
3) If a pathogen is identified as causing infection, then determine which therapies might be effective

Today’s objective

Apply the principles of antimicrobial susceptibility testing (AST) and bacterial resistance mechanisms to the testing and interpretation of bacterial isolates.

- Compare various AST principles and procedures by definition and methodology.
- Define terms related to interpretation of susceptibility tests
- Explain the use of the McFarland turbidity standard when preparing an inoculum for AST.
- Describe the standard quality control documents for performing antimicrobial susceptibility testing.
- Describe AST based on dilution methods.
- Describe disk diffusion testing (Kirby-Bauer test) parameters and troubleshooting issues.
- Describe the gradient diffusion test (Epsilometer test “Etest”).
- Distinguish the principles of antimicrobial mechanisms of resistance.

Biocides

Antimicrobial (aka antibiotic)

A chemical compound that selectively inhibits a vital metabolic process of a microbe (e.g. cell wall synthesis, DNA synthesis, protein synthesis) or a biological agent that selectively acts on a microbe of interest (e.g. bacteriophage, lysin) and is generally safe to administer to humans.

Disinfectant/Antiseptic

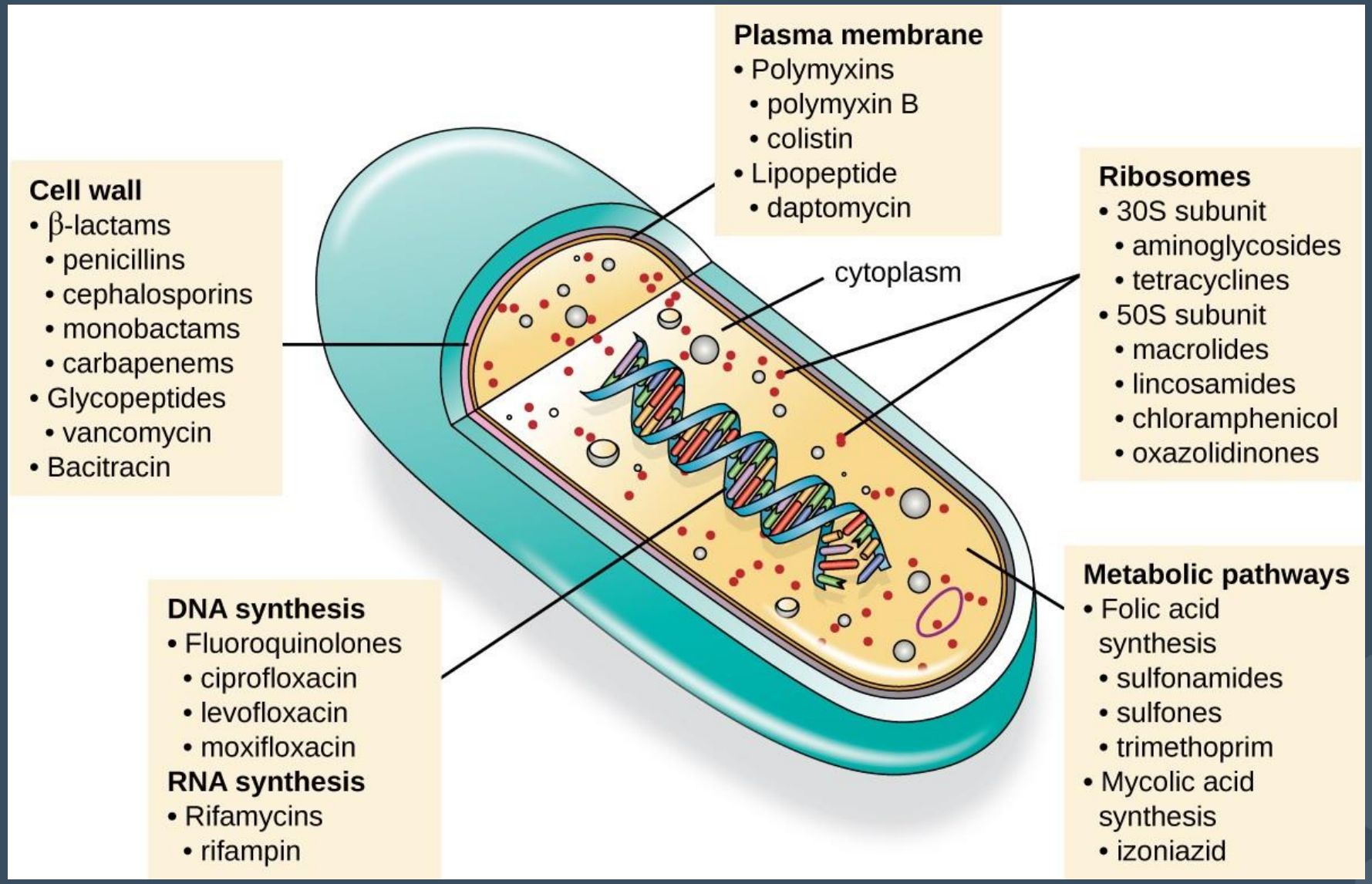
Typically used to decontaminate a surface. These are generally different from antibiotics in that they typically are chemicals that kill by contact, require higher concentrations, and are more toxic.

Inhibit		Classification					Antibiotics
Cell Wall SYNTHESIS	Beta Lactams	**Penicillins**	Penicillinase-Sensible				
			Natural Penicillins (Narrow Spectrum)	Penicillin G: Na, K, Procainic, Benzathine(IV, IM) Penicillin V: VO			
			Aminopenicillins (Broad Spectrum)	Ampicillin Amoxicillin			
			Penicillinase – Resistant (very narrow spectrum)				
			Nafcillin	Oxacillin	Dicloxacillin		
			Antipseudomonal (extended spectrum)				
			Carboxipenicillins	**Ticarcillin- Carbenicillin**			
			Ureidopenicillins	Azlocillin – Mezlocillin - Piperacillin			
		Cephalosporins	**1 st Generation**	Cefazolin	Cephalexine	Cephapirin	
				Cefadroxil	Cephadrine	Cephalotin	
			2 nd Generation	Cefuroxime	Cefoxitin	Cefotetan	
				Cefamandole	Cefonicid	Cefaclor	
				Cefprozil	Cefmetazole		
			3 rd Generation	Cefoperazone	Ceftriaxone	Ceftazidime	
				Cefpodoxime	Ceftizoxime	Cefotaxime	
				Cefdinir	Ceftibuten	Cefixime	
				Cefditoren			
			4 th Generation	Cefepime	Cefpirome		
			5 th Generation	Ceftaroline			
		Carbapenems	**Meropenem**	**Ertapenem**	**Doripenem**	**Imipenem + Cylastatine**	
		Monobactams	Aztreonam				
		Beta-lactamase inhib.	Sulbactam	Tazobactam	Clavulinic acid		
	No lactam	**Glycopeptides**	Vancomycin		Bacitracin		
			Teicoplanin		Polymyxin B		
Protein Synthesis		S30	Amino-glycoside	Gentamycin	Neomycin	Streptomycin	
				Amikacin	Tobramycin		
			Tetracyclins	Doxycycline	Demeclocyclin	Minocycline	
				Tetracyclin	Tigecyclin		
		S60	Oxazolidonones	Linezolid			
			Streptogramins	Quinupristin/Dalfopristin			
			Chloramphenicol				
			Macrolides	Erythromycin	Azithromycin	Clarithromycin	
			Lincosamides	Clindamycin	Lincomycin		
DNA toboisomerases		Fluoroquinolones	Ciprofloxacin	Norfloxacin	Levofloxacin	Ofloxacin	
			Sparfloxacin	Moxifloxacin	Gemifloxacin	Enofloxacin	
		Quinolones	Nalidixic Acid				
Folic Acid Synthesis		Sulfonamides	Sulfamethoxazole (SMX)	Ag Sulfadiazine	Sulfasalazine	Sulfisoxazole	
		DHFR inhibitors	Trimethoprim		Pirymethamine		
DNA (damage)		Metronidazole					
mRNA synthesis		Rifampin					

http://i.imgur.com/Vu6MG9K.png

Mechanisms of resistance

Mechanisms of Action



https://courses.lumenlearning.com/microbiology/chapter/mechanisms-of-antibacterial-drugs/

Mechanisms of Resistance

- The intracellular concentration of the antibiotic is reduced by increased efflux or porin mutation/loss.
- The bacterium produces an enzyme that inactivates the antibiotic.
- The antibiotic target is modified, replaced, or overproduced; so that the antibiotic no longer interferes with the bacterium’s metabolism.

Ways to describe resistance

Expression mediated resistance

- Enzyme upregulation (e.g. DHFR)
- Efflux upregulation (e.g. *Pseudomonas*)
- Porin downregulation (e.g. *Pseudomonas*)
- Biofilm/persister phenotype
  (e.g. device-associated infections)

Genetically mediated resistance

- Intrinsic (e.g. *Stenotrophomonas*)
- Spontaneous mutation (e.g. *rpoB*)
- Acquired (e.g. bla_{KPC})

Acquisition of genetic elements

Genetic elements of resistance can be acquired by

- transformation (ie DNA from the environment)
- transduction (ie phage)
- conjugation (eg plasmids)

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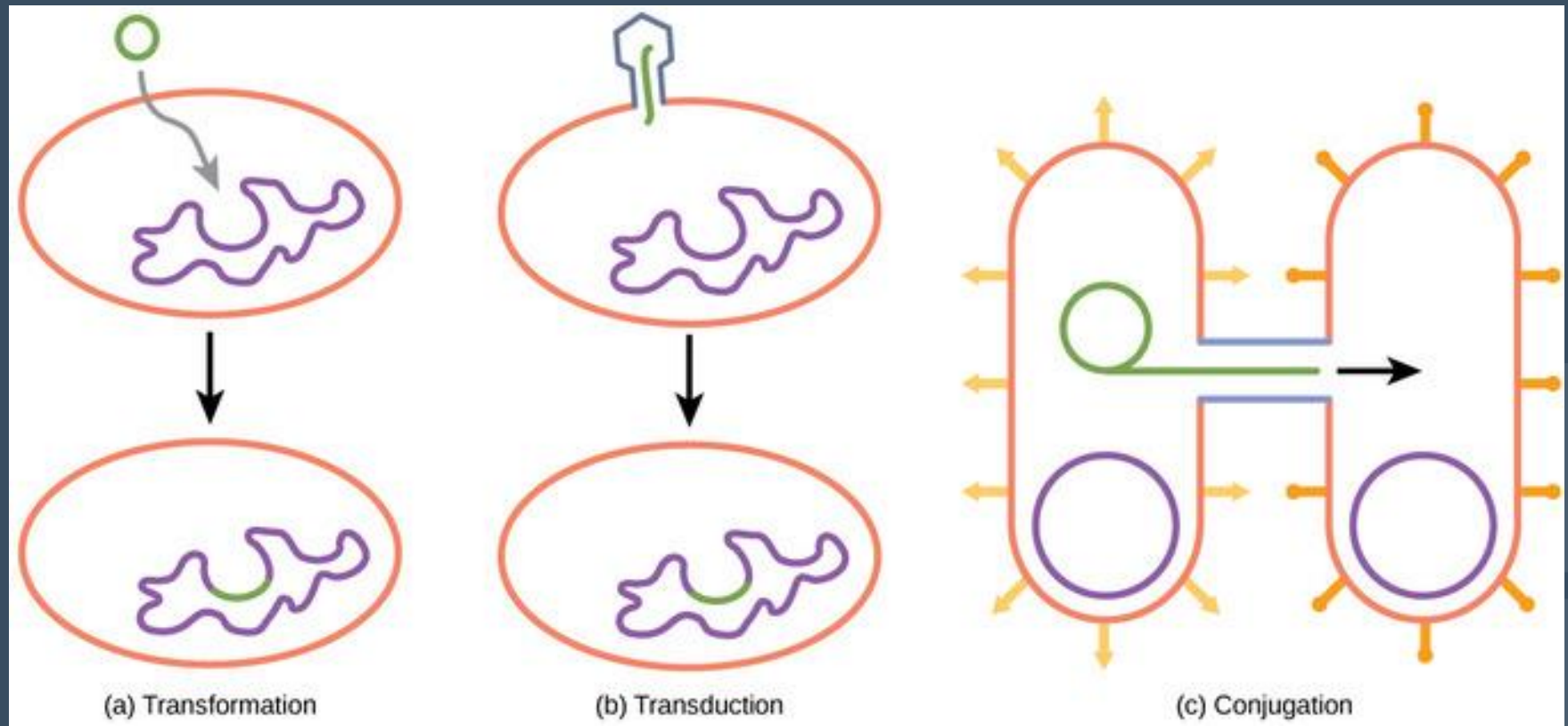
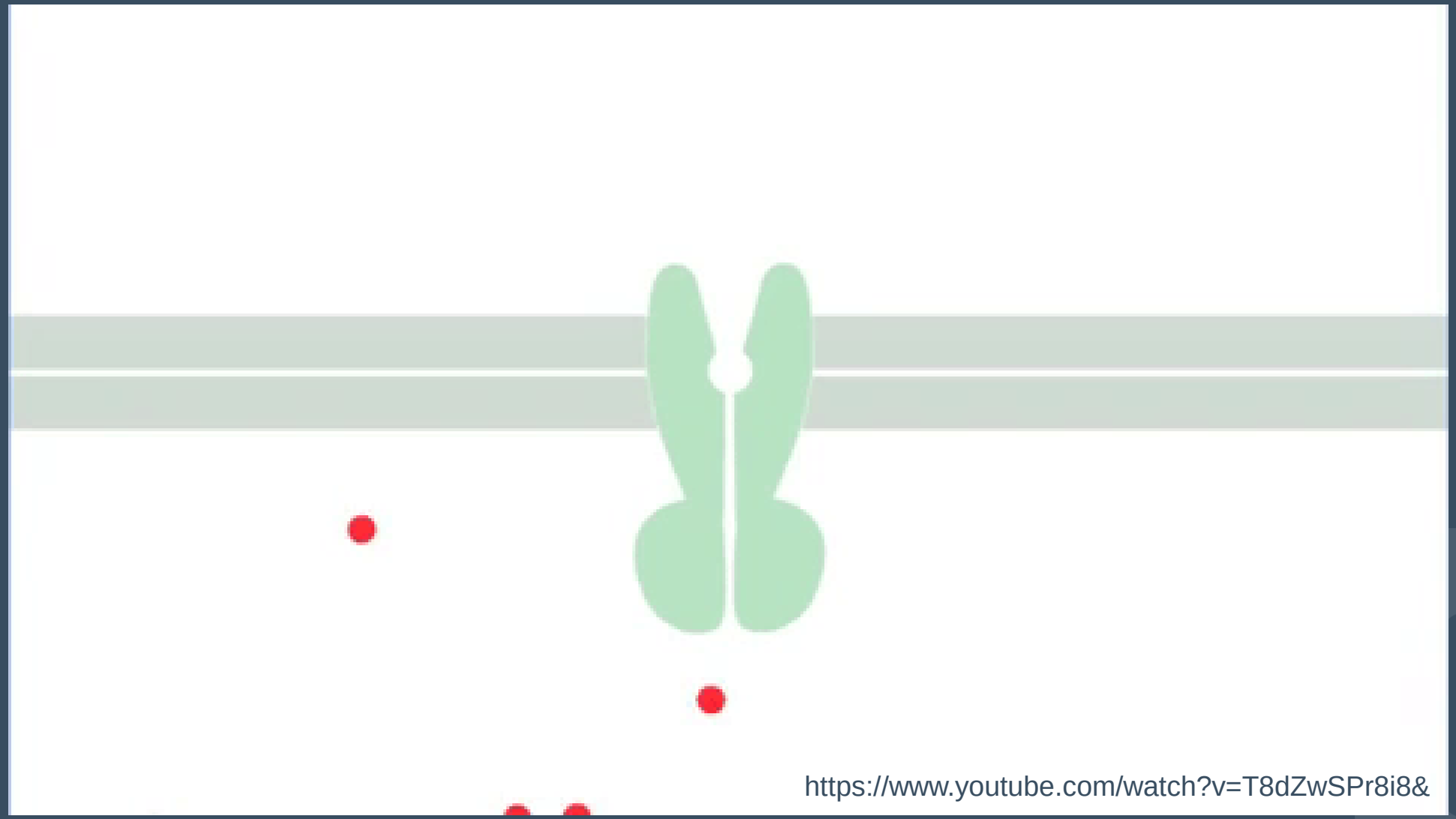


Efflux upregulation & porin downregulation

Resistance to many antibiotics is achieved, at least in part, through manipulation of the proteins that enable substances to enter the cell and which pump substances out of the cell.

Efflux upregulation
& porin downregulation

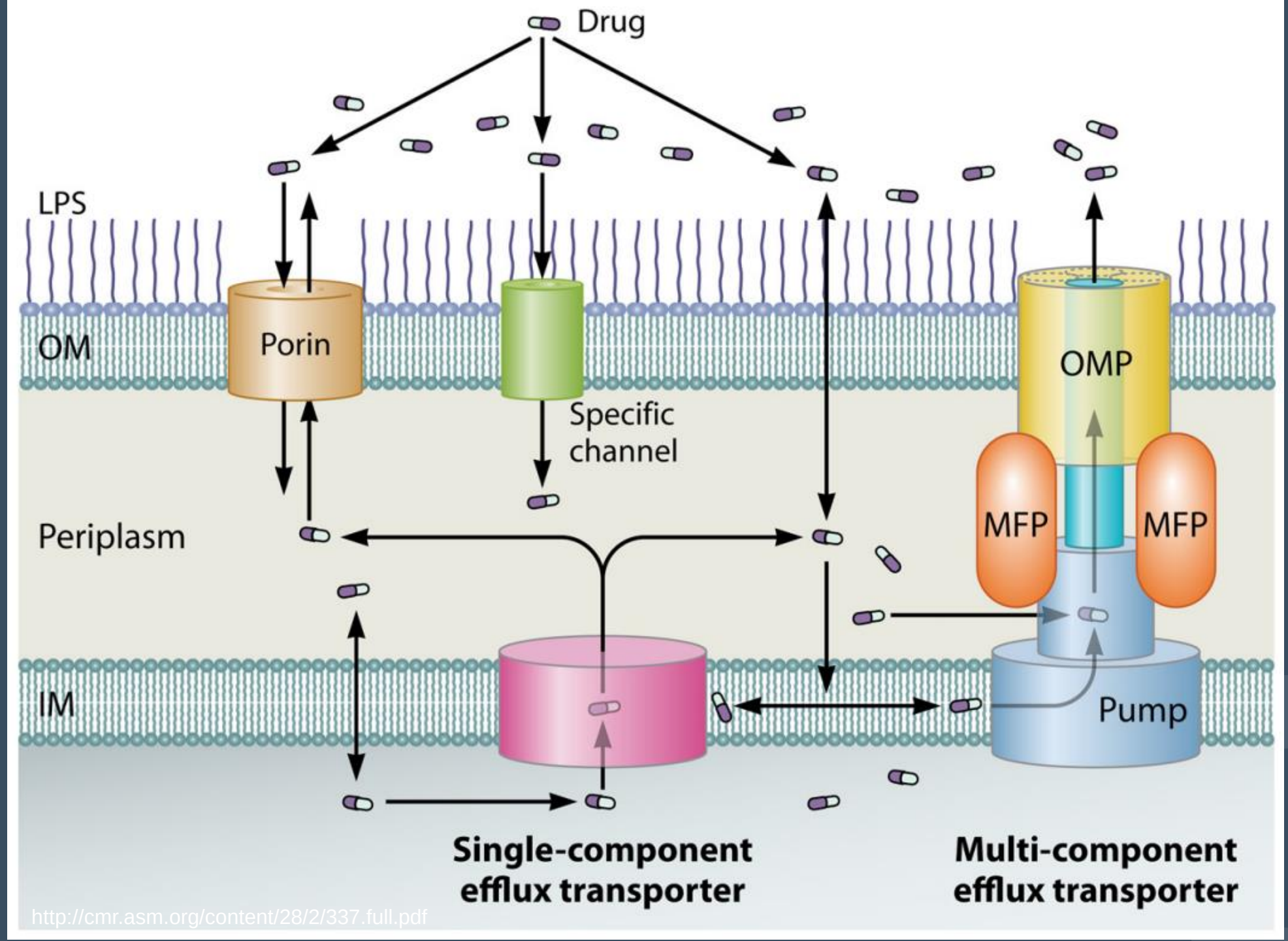


https://www.youtube.com/watch?v=T8dZwSPr8i8&

Efflux upregulation & porin downregulation

"*P. aeruginosa* possesses specific channels, such as OprB, specific for glucose uptake, and OprD, specific for the diffusion of basic amino acids and peptides. [OprD] is the primary channel for the entry of carbapenems across the OM, and the reduced expression or loss of OprD has been frequently observed in carbapenem-resistant clinical isolates, which may also display upregulated drug efflux systems"

http://cmr.asm.org/content/28/2/337.full.pdf



http://cmr.asm.org/content/28/2/337.full.pdf

Antibiotics

Beta lactams
Vancomycin
Daptomycin
Linezolid
Macrolide / Lincosamide / Streptogramin
Rifampin
Fluoroquinolone
Aminoglycosides
Tetracyclines
Trimethoprim / sulfamethoxazole

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Penicillin

THE NEW LIFE-SAVING DRUG

Saves Soldiers' Lives!

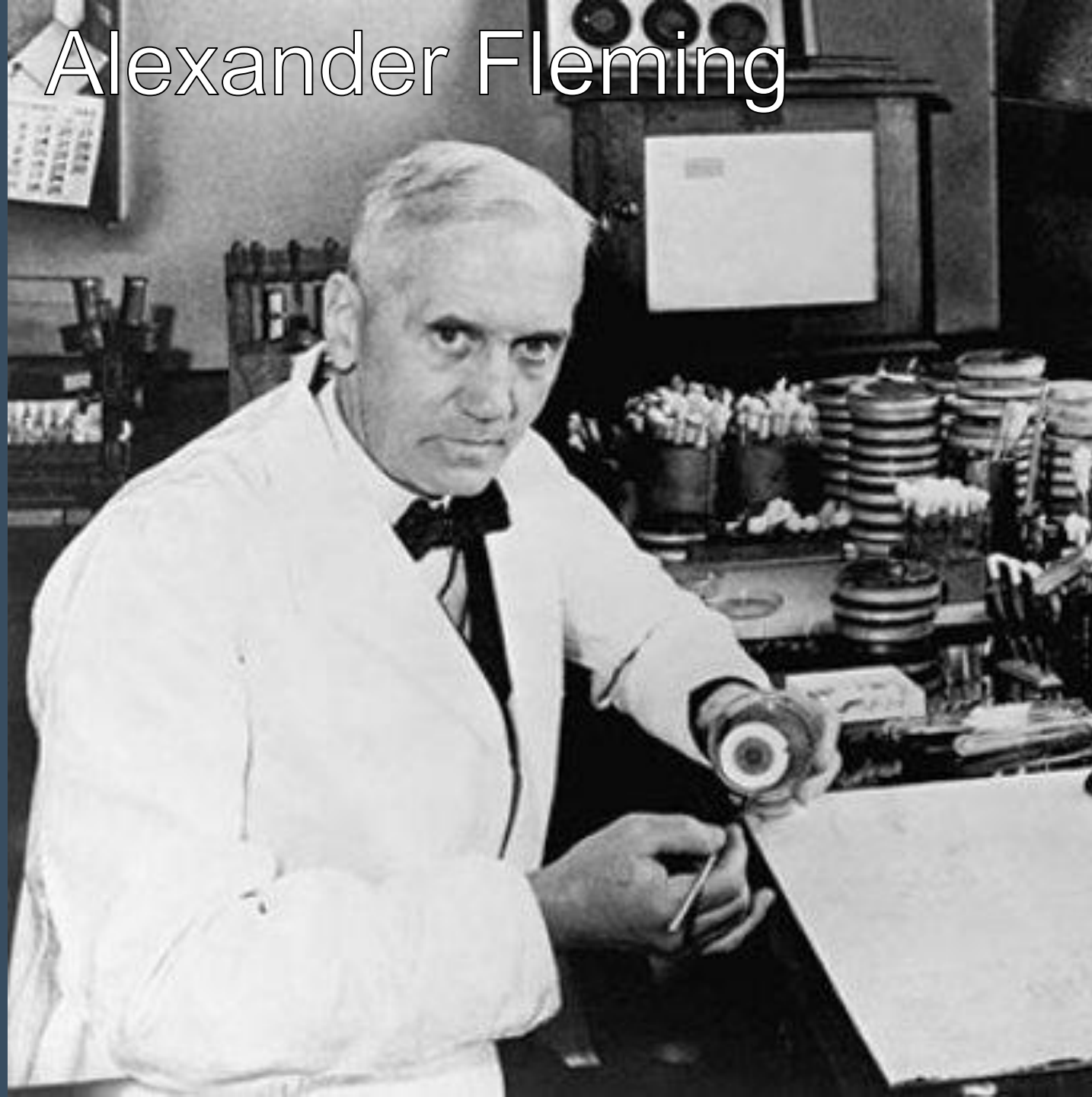
Men who might have died will live...if YOU

Give this job Everything You've got!

Alexander Fleming

"[H]e found that a mould culture prevented growth of staphylococci, even when diluted 800 times."

From *Nobel Lectures, Physiology or Medicine 1942-1962*, Elsevier Publishing Company, Amsterdam, 1964.



One ring to rule them all

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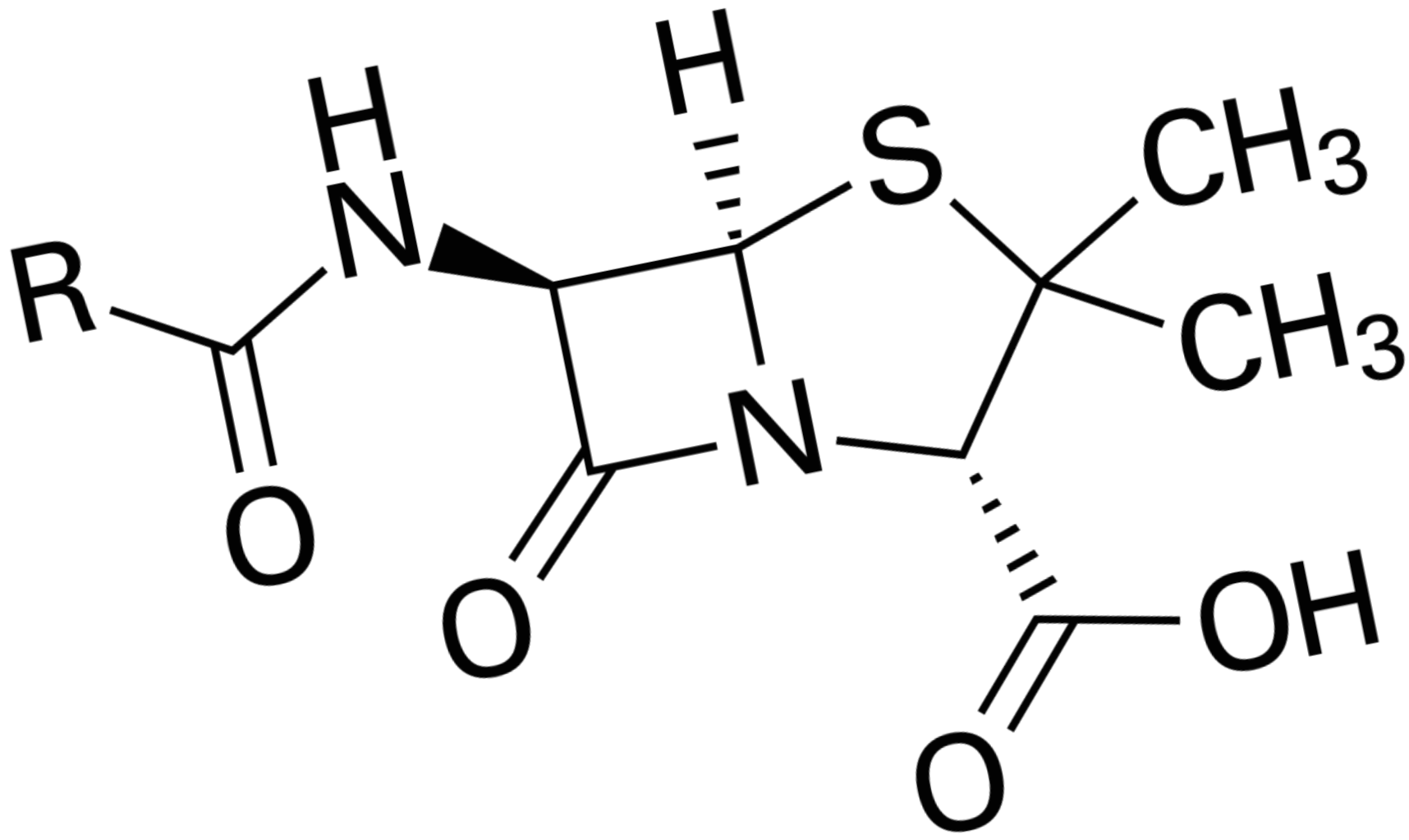
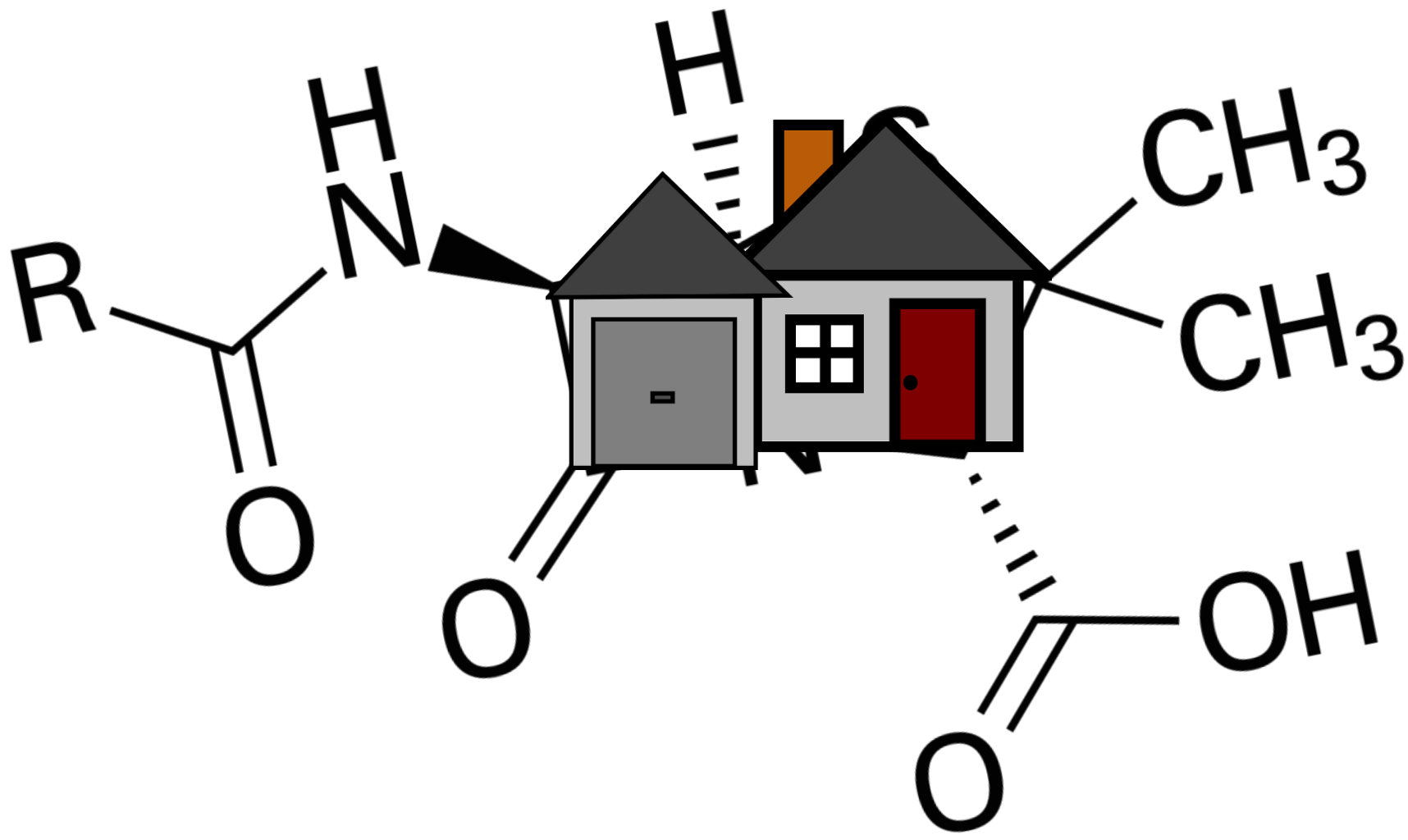
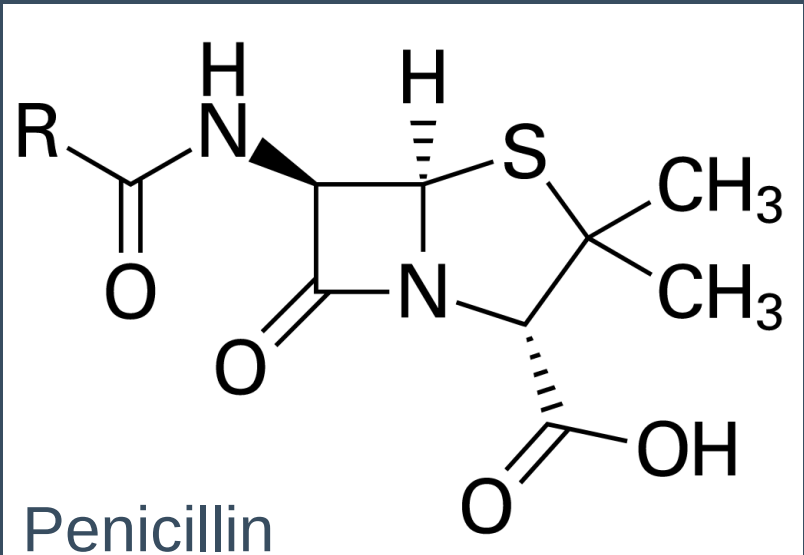


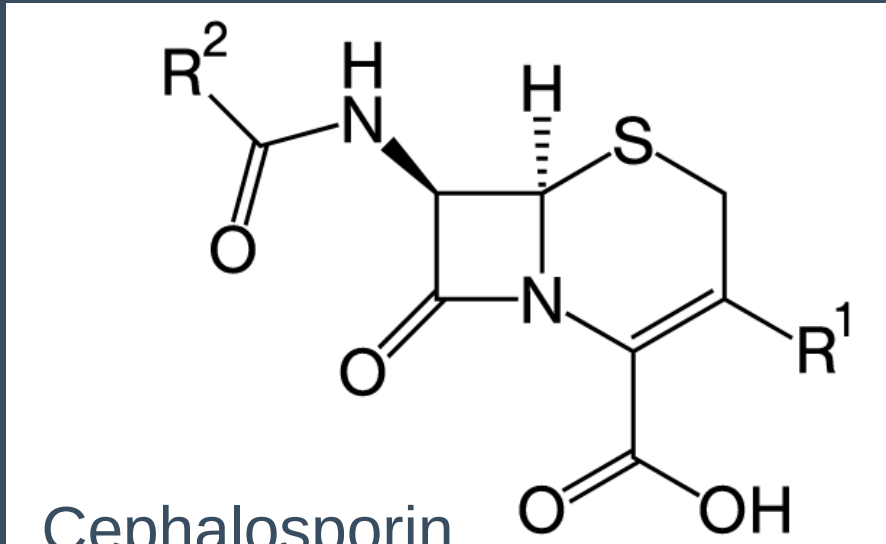
One ring to rule them all



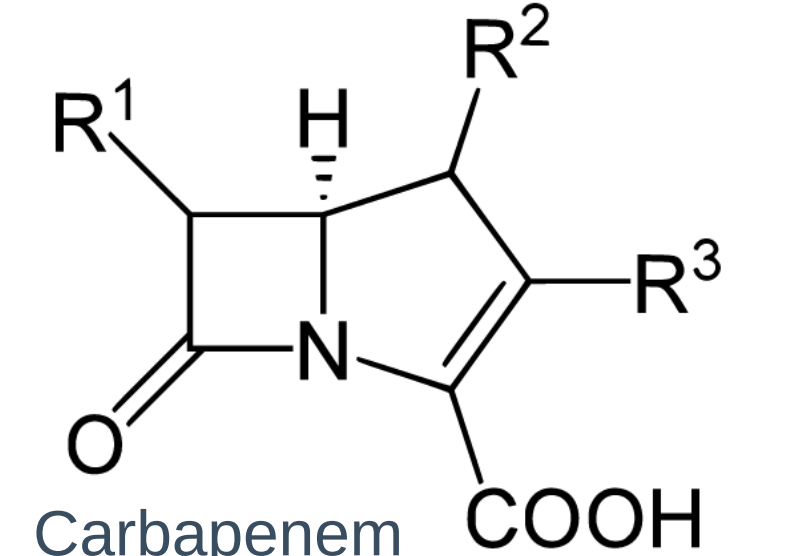
One ring to bind them



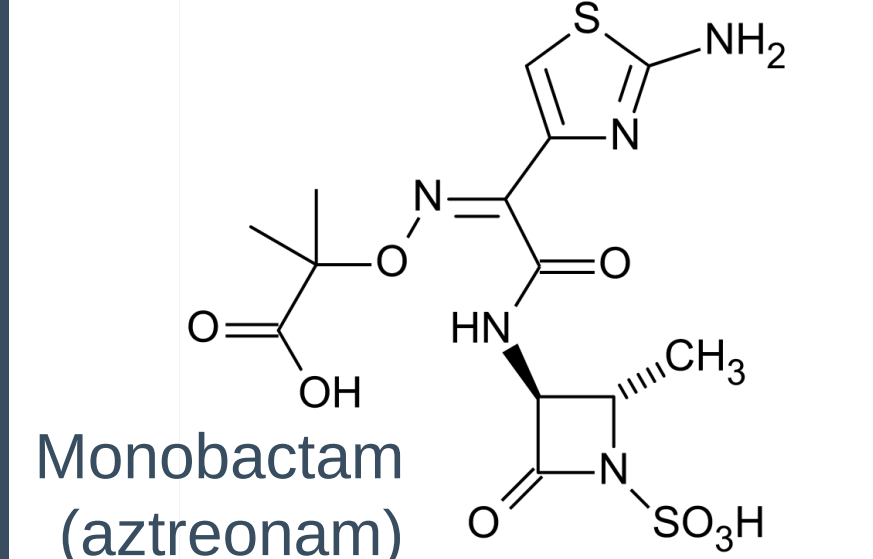
Penicillin



Cephalosporin



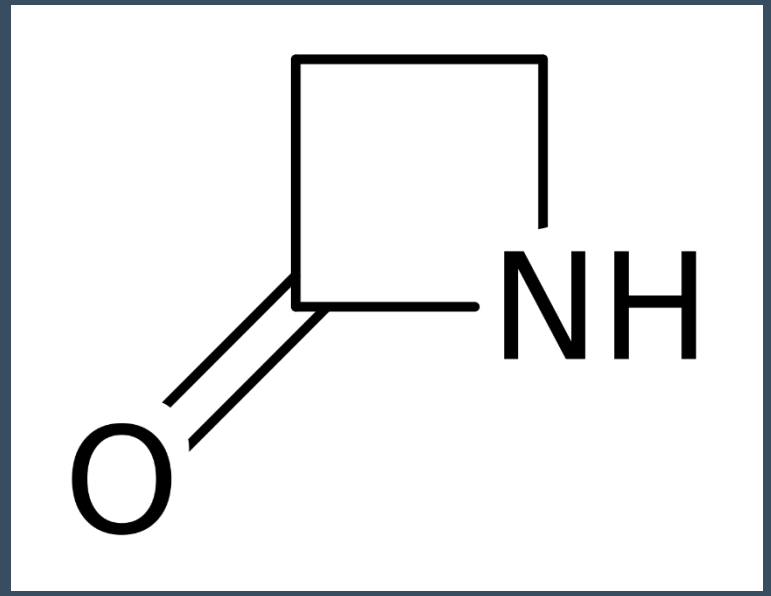
Carbapenem



Monobactam (aztreonam)

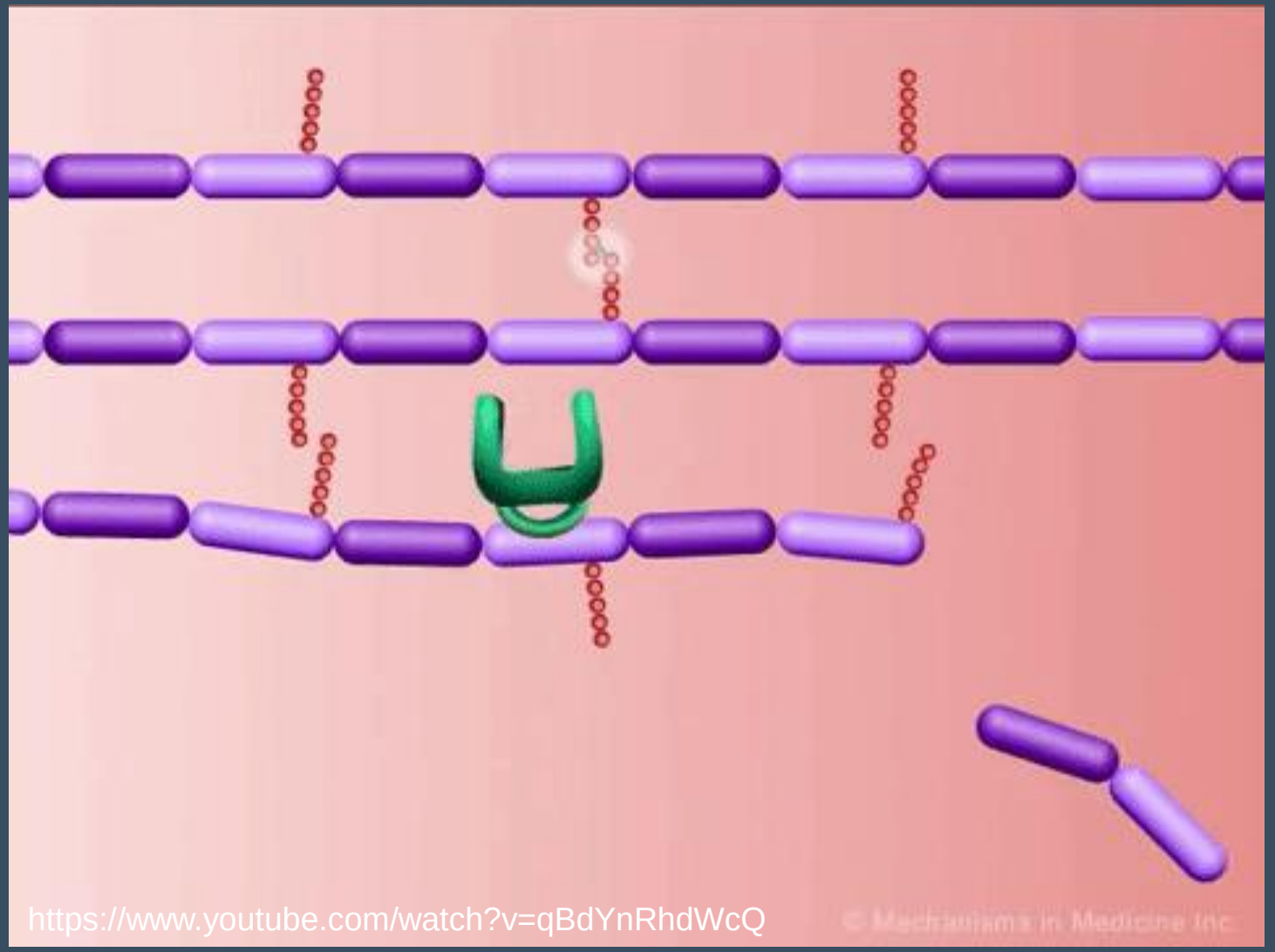
The beta lactam ring

The beta-lactam ring acts as competitive inhibitor of penicillin binding proteins (PBPs). PBPs are transpeptidases involved in cross-linking the peptidoglycan cell wall.



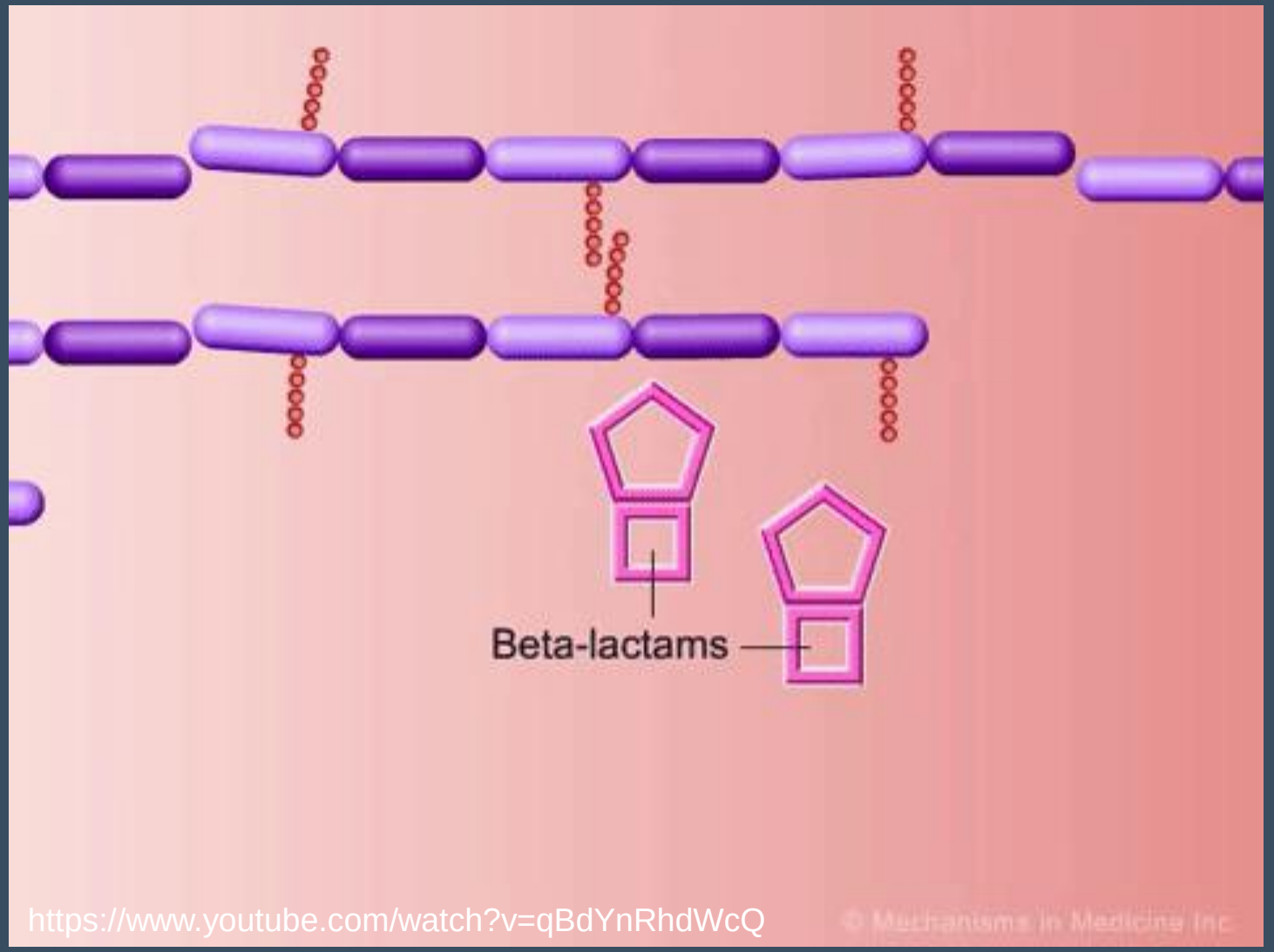
The beta lactam ring

Peptidoglycan is cross-linked by PBP.

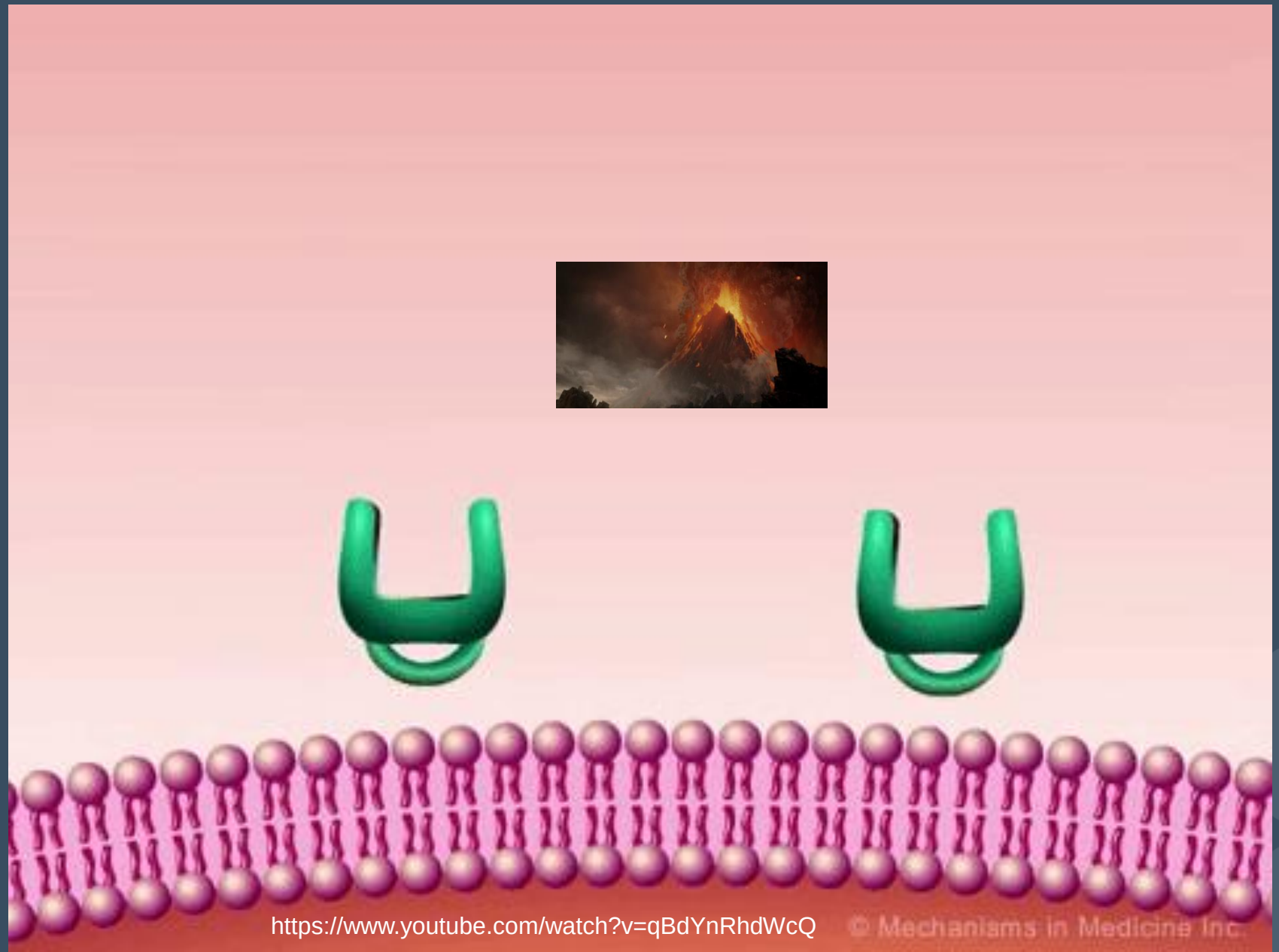


The beta lactam ring

Beta-lactams inhibit unaltered PBP.



The beta lactam ring



Beta lactamase reaction

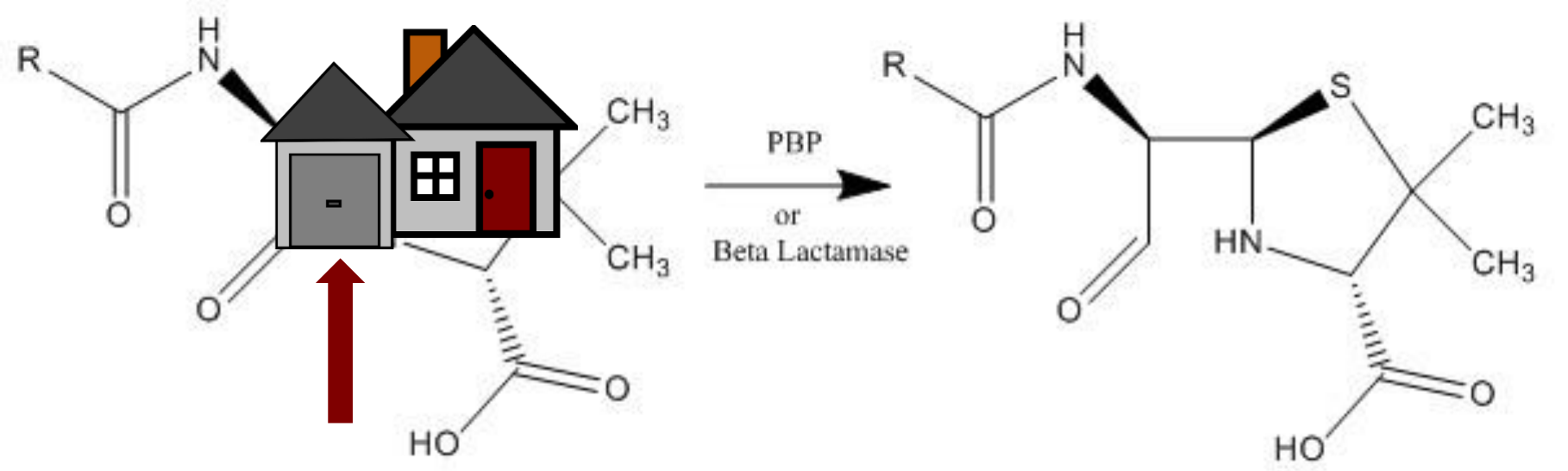
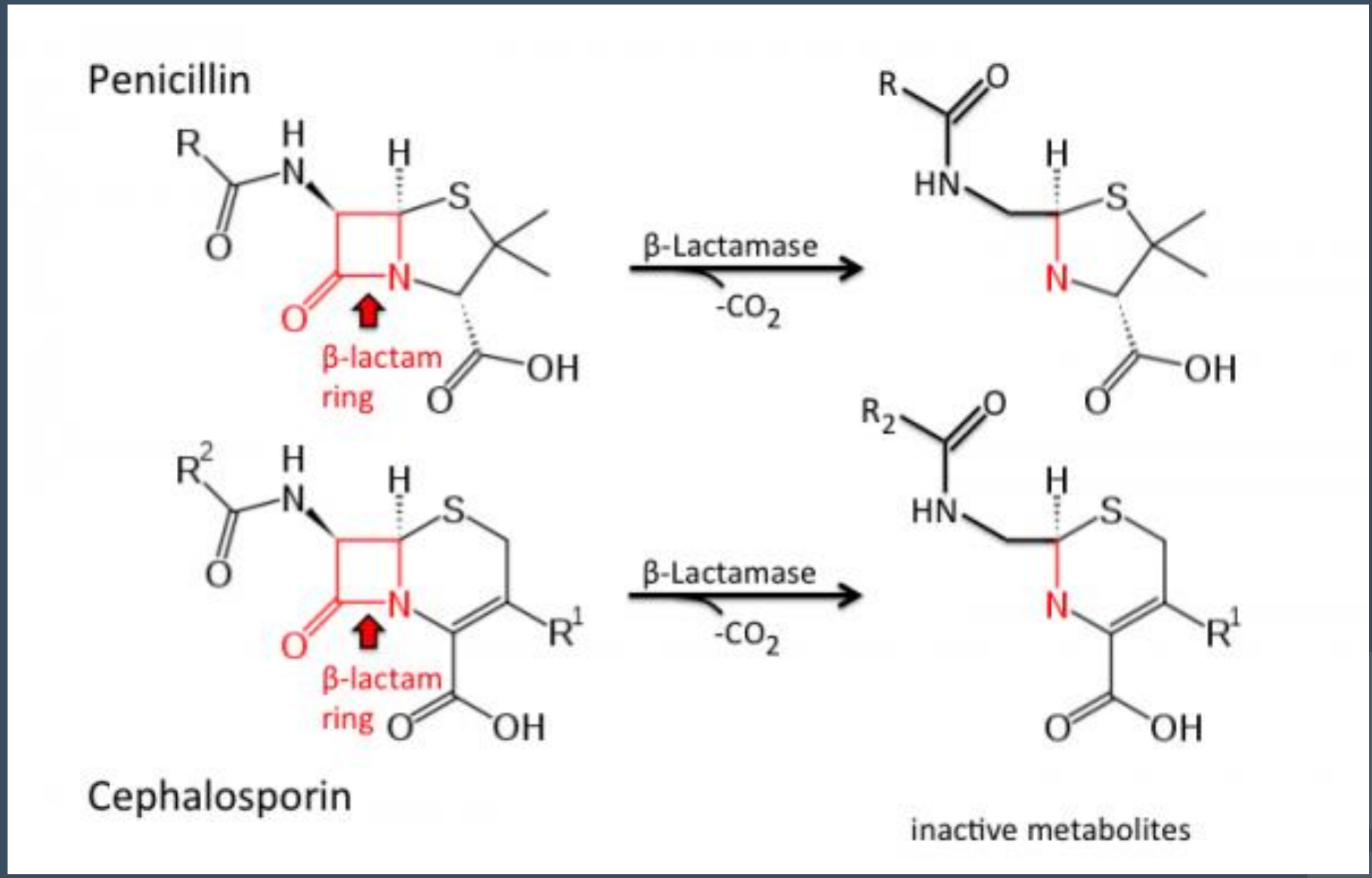


Figure (3) Breaking of the beta lactam ring

http://chemistry.berea.edu/~biochemistry/2013/uo_at/Figure2.jpg

Beta lactamase reaction



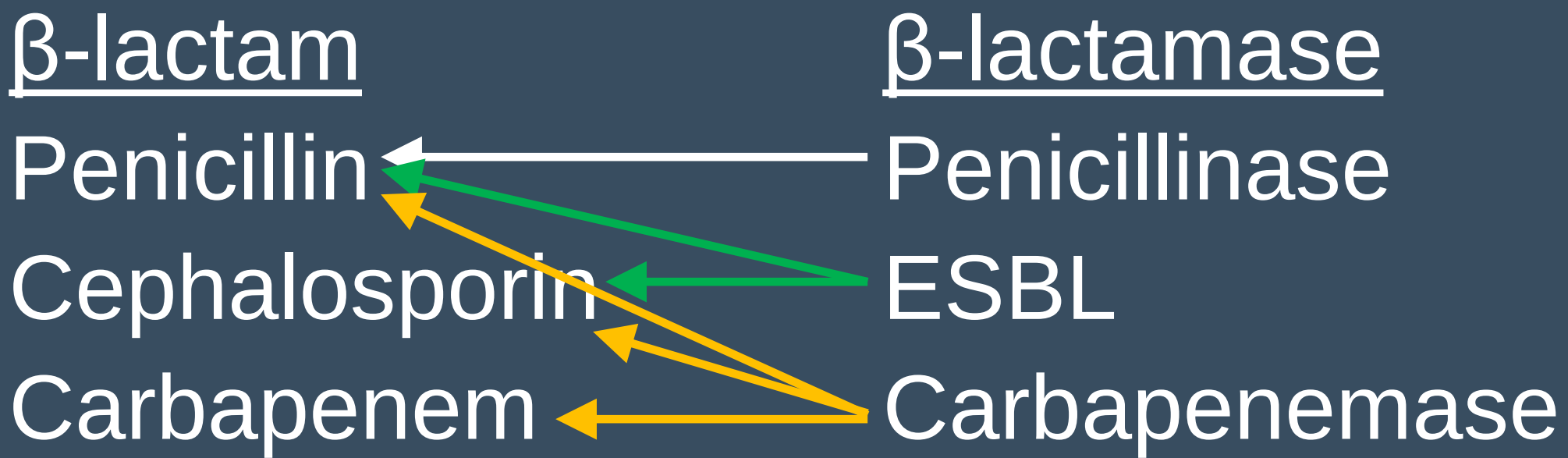
http://tmedweb.tulane.edu/pharmwiki/lib/exe/fetch.php/betalactams.png?w=600&tok=3379e1

β-lactams & β-lactamases

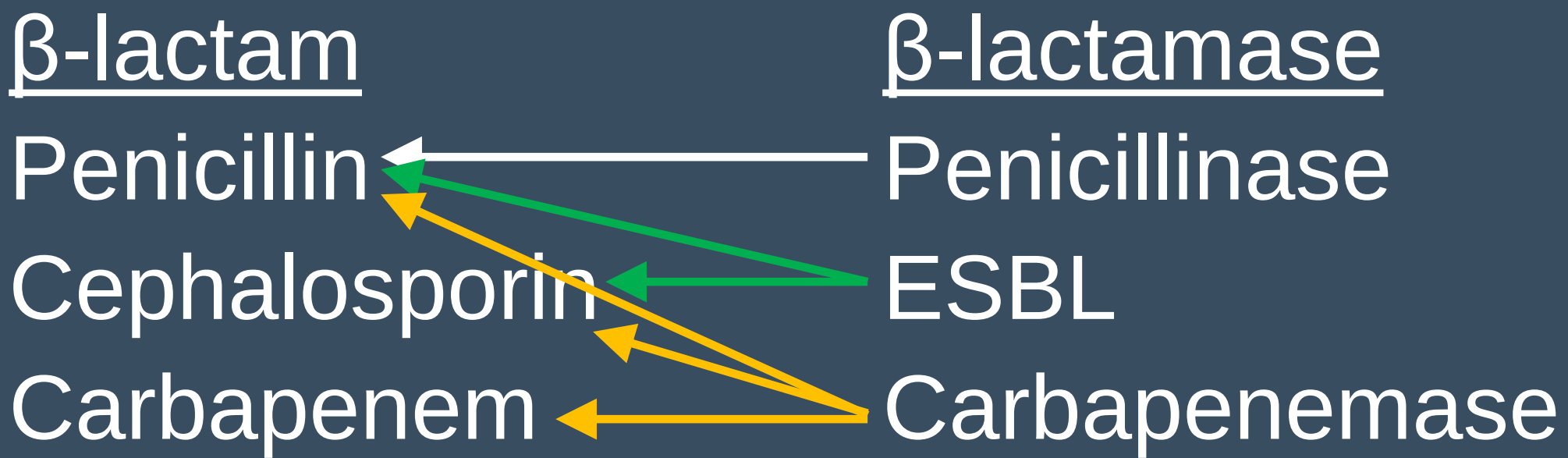
Beta-lactamases can be described in several ways:

- substrates which can be hydrolyzed
  (spectrum of activity)
- level of gene expression
- structure (serine vs zinc at the active site)
- acquired vs intrinsic

β-lactams & β-lactamases



β-lactams & β-lactamases



β-lactams & β-lactamases

Penicillinases

- *Bacteroides* spp. and other gram negative anaerobes
- Many *Bacillus* species (although not *B. anthracis*)
- Many fastidious “-ella” Gram negatives can have penicillinases: *Moraxella*, *Pasteurella*, *Eikenella*, *Haemophilus*
- Many *Staphylococcus* spp. & some *Enterococcus* spp.
- Penicillinases hydrolyze penicillins but not cephalosporins or carbapenems.

β-lactams & β-lactamases

Extended-spectrum beta-lactamase (ESBL)

- ESBLs can be acquired by *Enterobacterales*, most commonly *E. coli* and *Klebsiella*.
- CTX-M is most common, but SHV and TEM enzymes can also be ESBLs
- ESBLs hydrolyze penicillins and cephalosporins but not carbapenems.

β-lactams & β-lactamases

Carbapenemases

- Carbapenemases can be acquired by some gram-negative bacteria
  - *Enterobacterales* (e.g. KPC, NDM, OXA-48)
  - *Pseudomonas aeruginosa* (e.g. VIM)
  - *Acinetobacter baumannii* (e.g. OXA-23)
- Some gram-negative bacteria have intrinsic resistance to carbapenems due to carbapenemases (e.g. *Stenotrophomonas*)

β-lactams & β-lactamases

Initialisms & acronyms

- CRE: “carbapenem-resistant *Enterobacterales*”
  CRE is often inferred to possess a carbapenemase, but this is not absolutely true
- CP-CRE: “carbapenemase producing CRE”
- CRAB: “carbapenem-resistant *Acinetobacter baumannii*”
  CRAB is almost always due to OXA-23

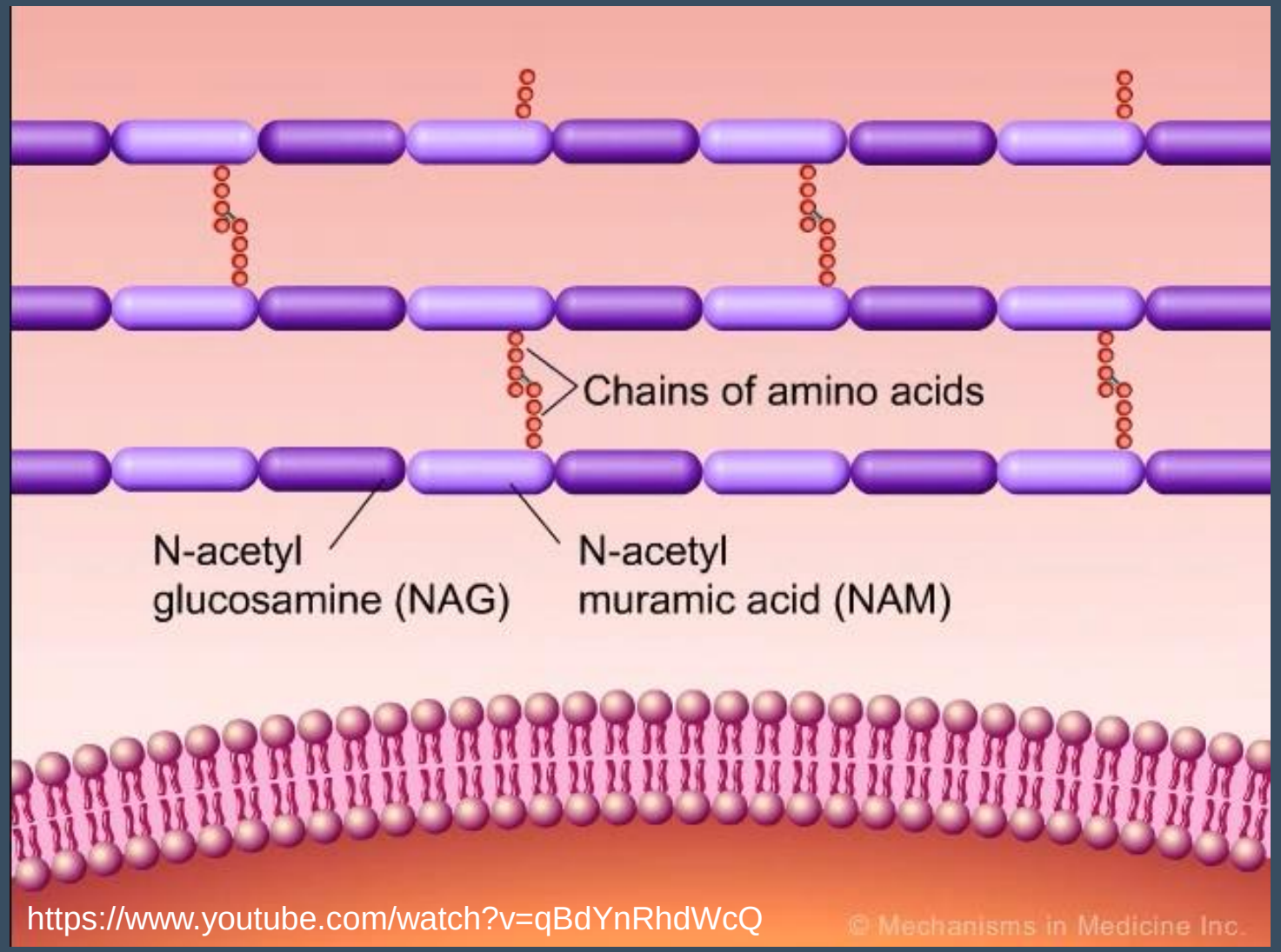
Methicillin-resistant staphylococci

Methicillin-resistant staphylococci

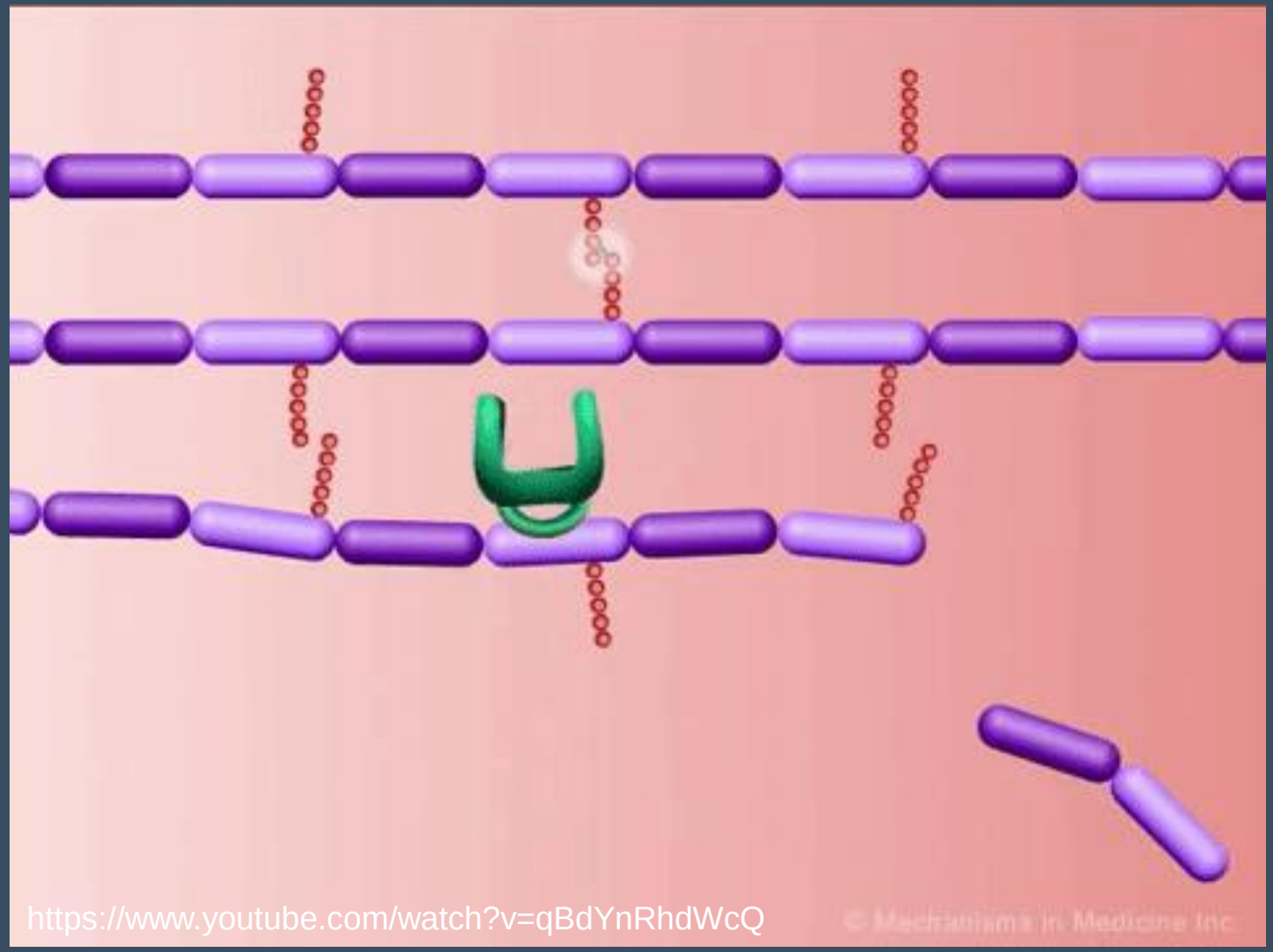
Isolated penicillin/ampicillin resistance in staphylococci is due to a penicillinase (i.e. enzymatic neutralization of antibiotic) that is common in many staphylococci.

“Methicillin resistance” in staphylocci is due to modification of the antibiotic’s target through acquired resistance.

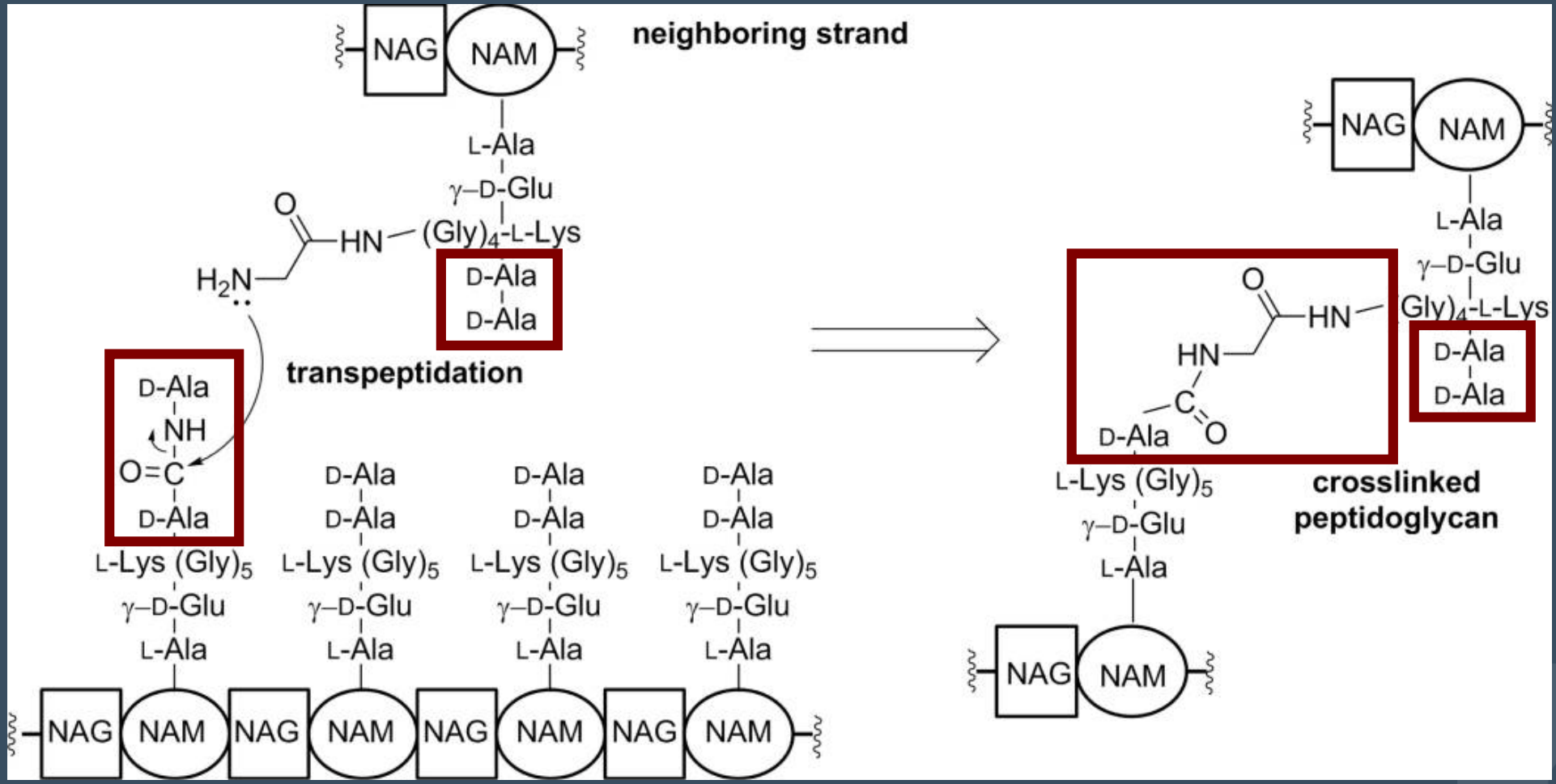
Peptidoglycan in staphylococci



Peptidoglycan synthesis

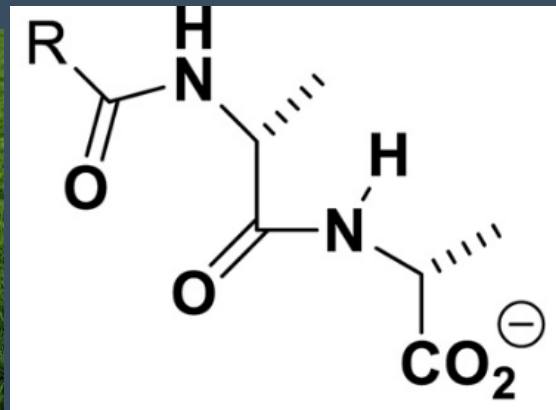


Peptidoglycan synthesis

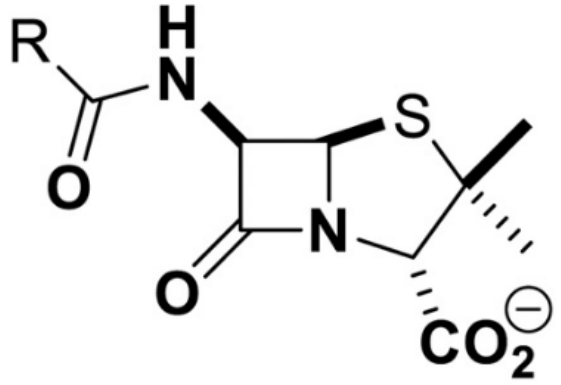


https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4236225/

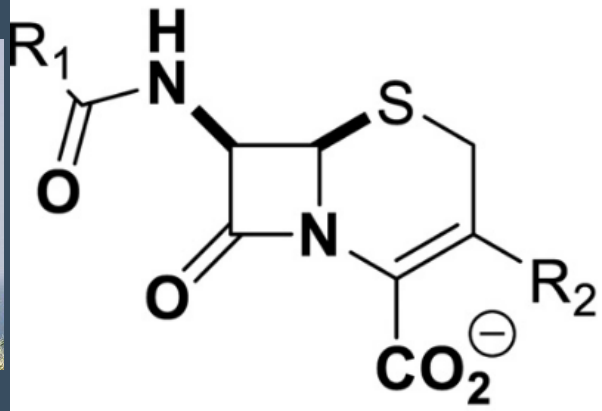
PBP substrate and β-lactam similarity



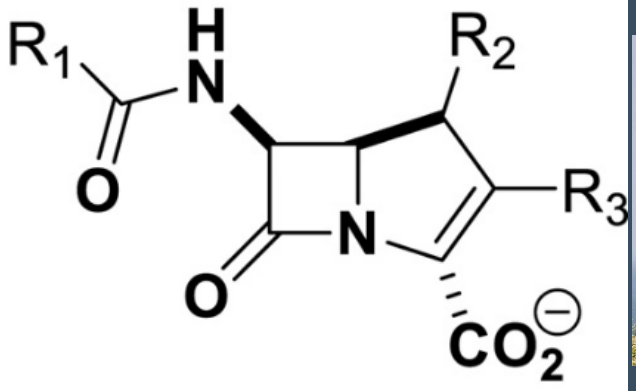
acyl-D-Ala-D-Ala



penicillin



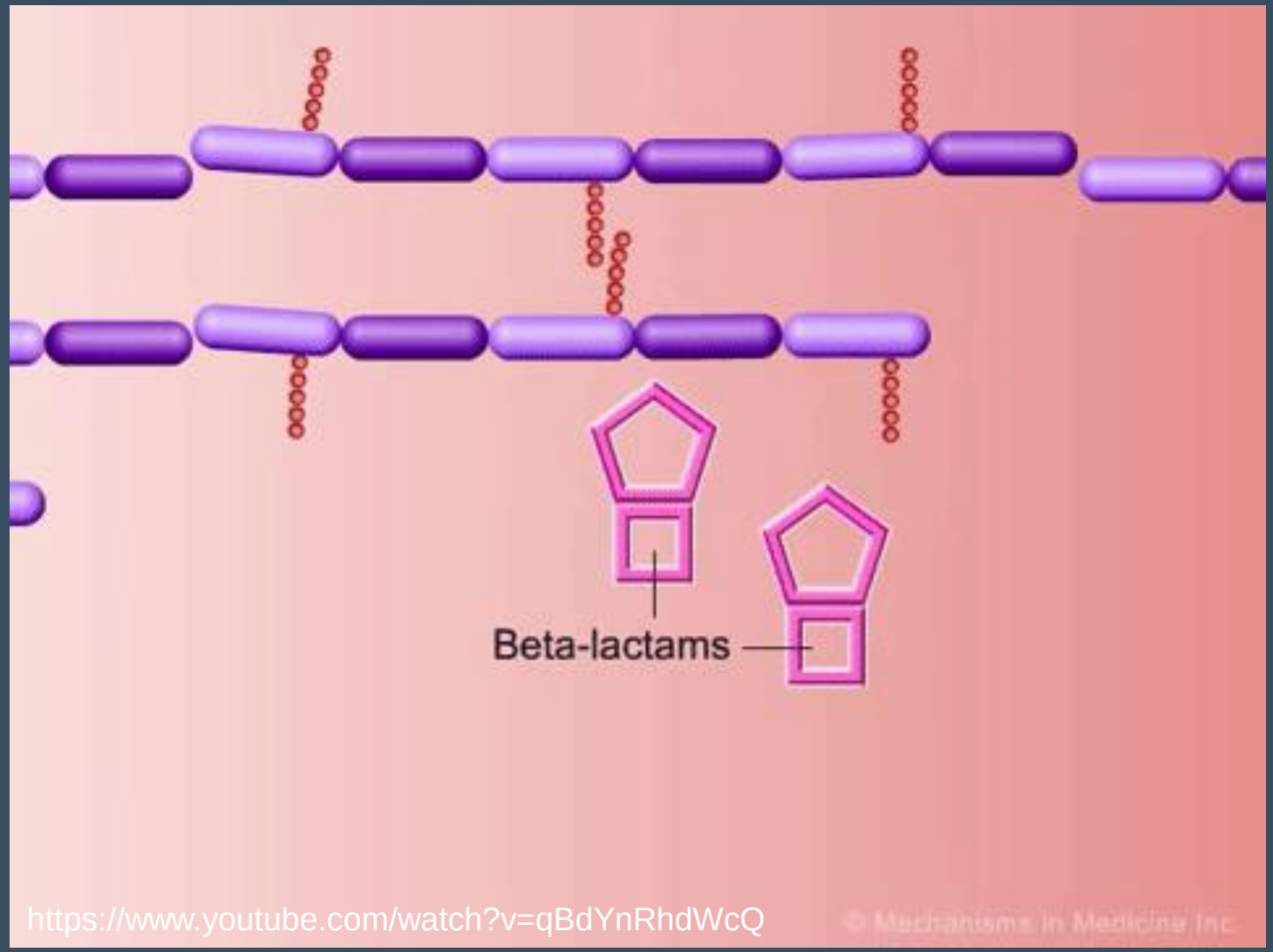
cephalosporin



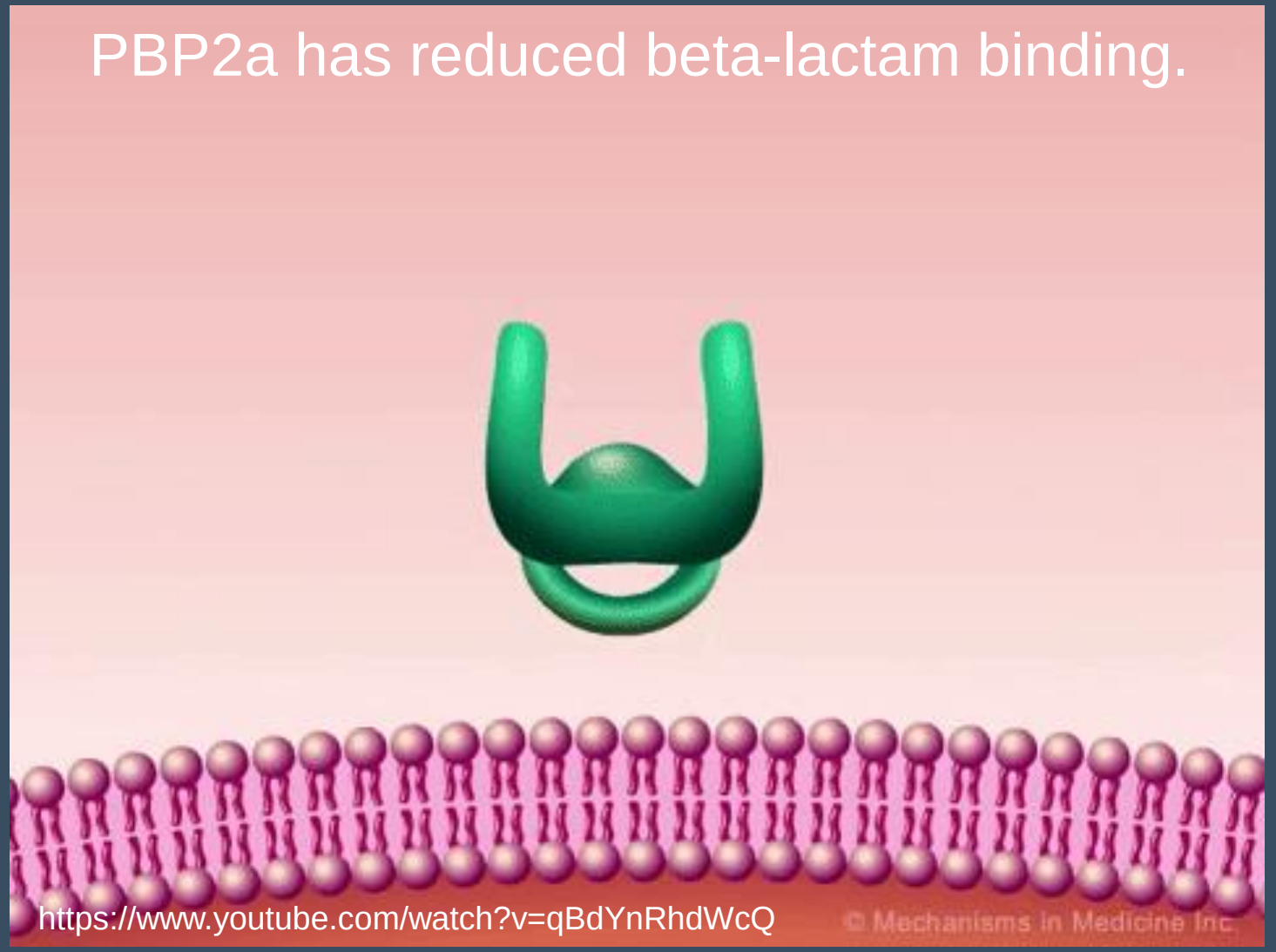
carbapenem

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4236225/

β-lactam activity in staphylococci



Methicillin-resistant staphylococci



Beta-lactams

Mechanism of action (MOA):
Interferes with peptidoglycan synthesis

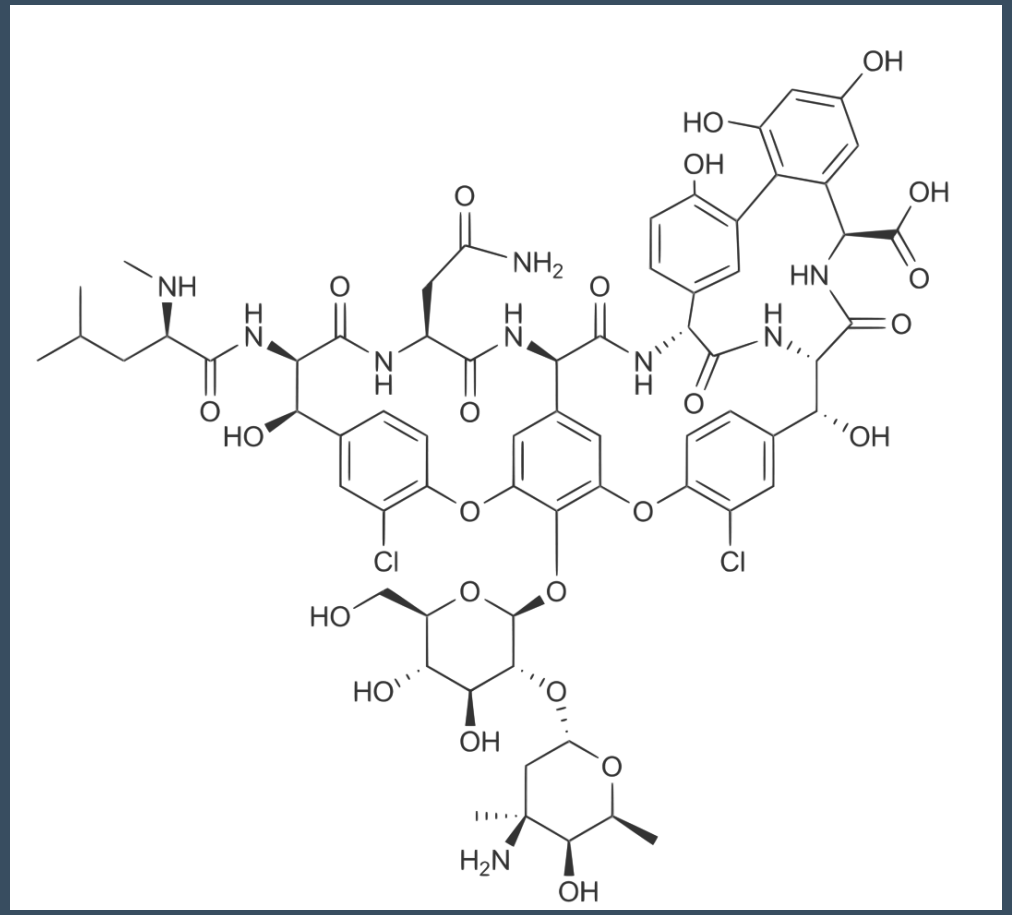
Target bacteria: Gram-positive & negative bacteria

Mechanism of resistance (MOR):
1) Increased efflux & decreased porins
2) PBP modification (e.g. MRSA)
3) Enzymatic inactivation (e.g. penicillinase).

Resistance to non-β-lactams

Vancomycin

Vancomycin is produced by *Amycolatopsis orientalis.*
It is a “glycopeptide” class of antibiotics.

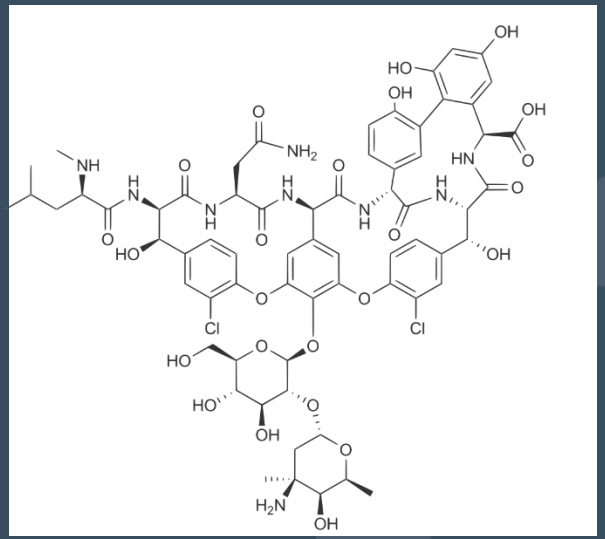


https://www.ncbi.nlm.nih.gov/pubmed/23814036

Vancomycin

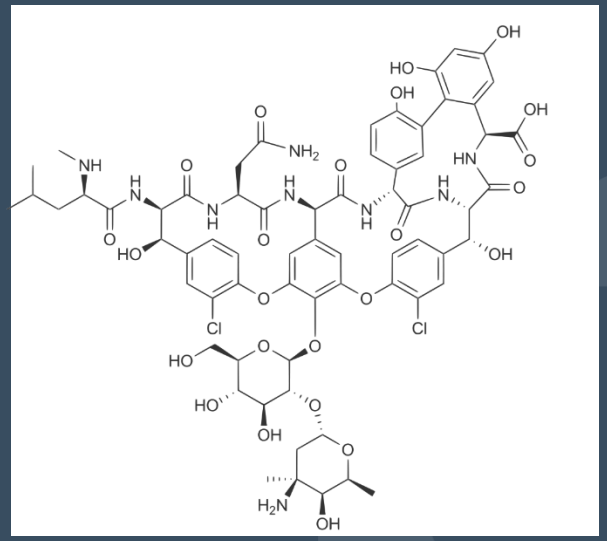
MOA:
Vancomycin interferes with peptidoglycan synthesis by binding to the peptide side chains, which are typically the substrate that PBPs crosslink.

Target bacteria:
gram-positive bacteria



Vancomycin

MOR:
In enterococci, vancomycin resistance can occur if the peptidoglycan pentapeptides do not have alanine-alanine termini. In staphylococci, vancomycin resistance is rare and has been linked to exaggerated cell wall thickness.



Vancomycin resistance

Enterococcus spp.

Typical peptidoglycan structure:

D-Ala-D-Ala

vanA: D-Ala-D-Lac

vanB: D-Ala-D-Lac

vanC: D-Ala-D-Ser

http://cmr.asm.org/content/13/4/686.full.pdf

D-Ala-D-Ala D-Ala-D-Ser D-Ala-D-Lac

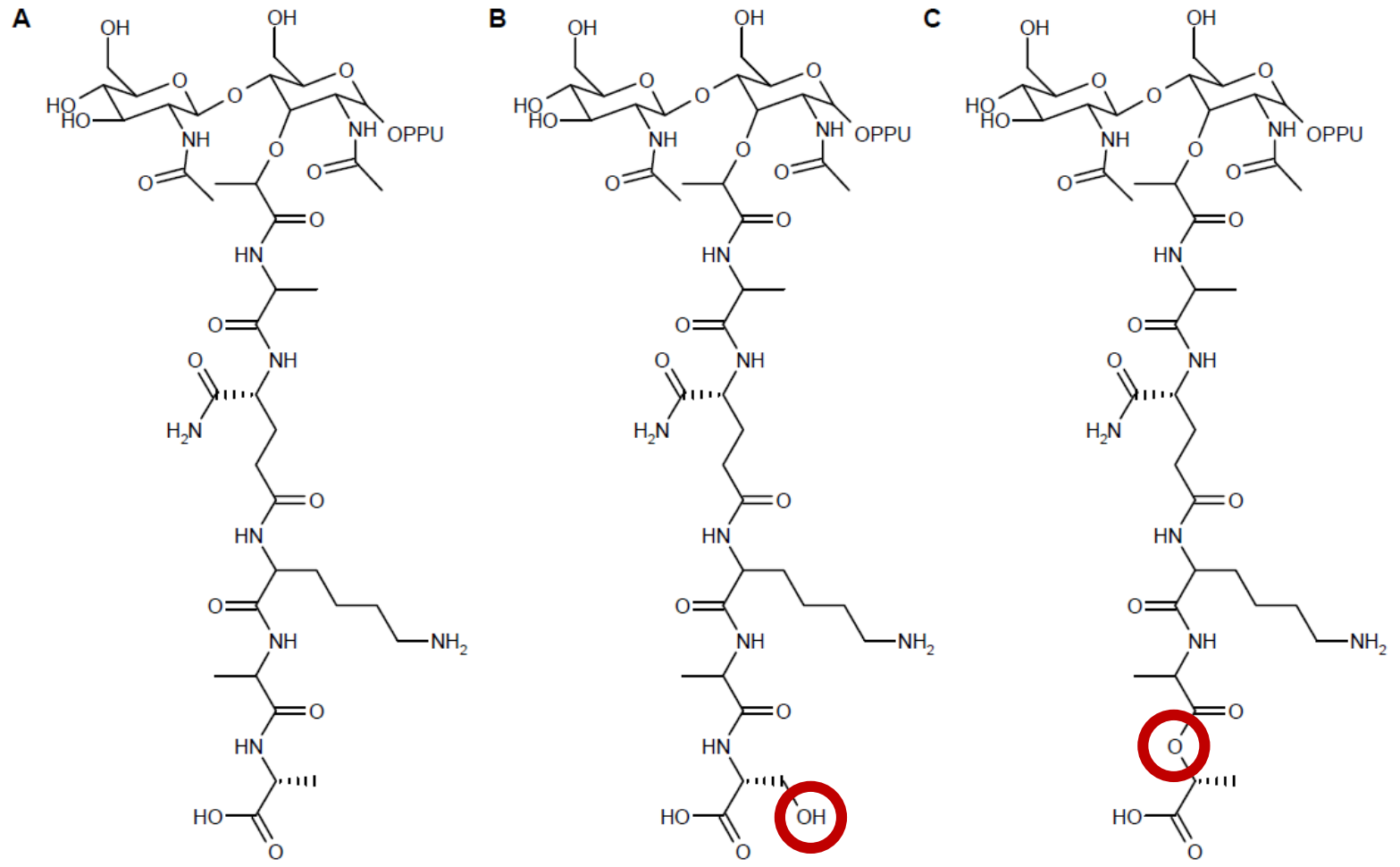
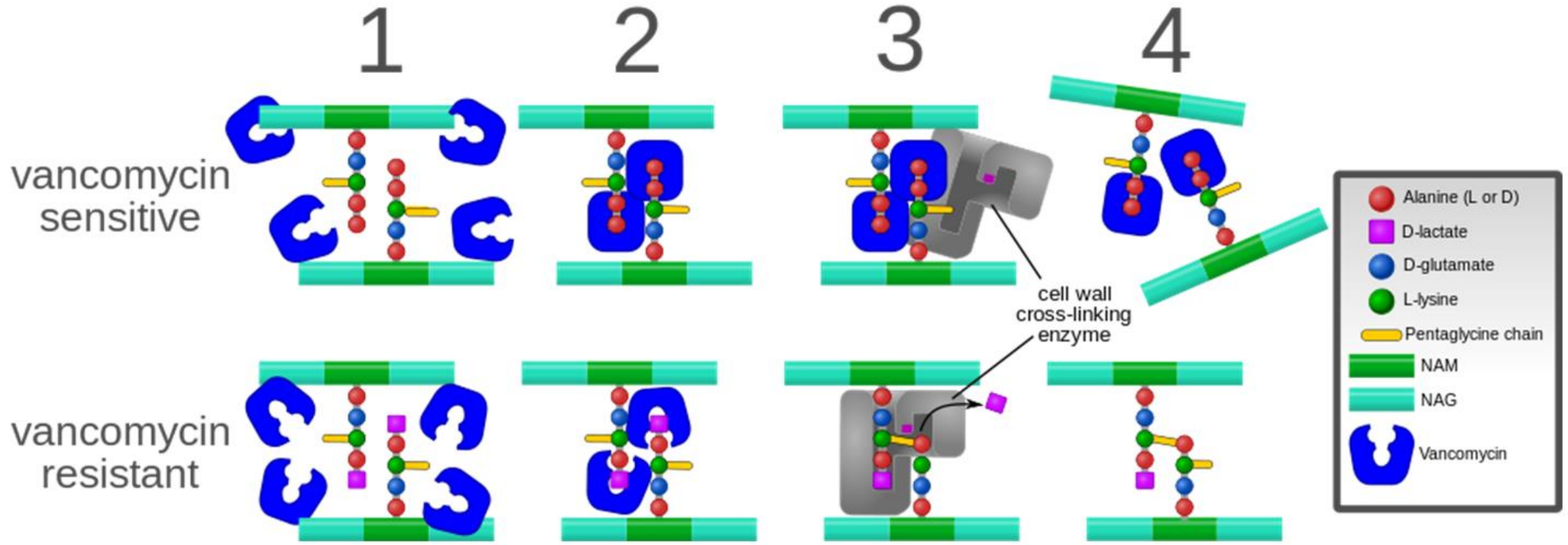


Figure 3. Structure of the PG precursors. The predominant precursor undecaprenyl-pyrophosphate-*N*-acetylmyramyl(*N*-acetylglucosamine)-L-Ala-D-Gln-L-Lys-D-Ala-D-Ala is illustrated in **A. B** and **C** represent the D-Ala-D-Ser and D-Ala-D-Lac variants, respectively, synthesised by VRE. PPU represents the undecaprenyl-pyrophosphate moiety of the lipid II precursor that is used for PG assembly.
PG: Peptidoglycan; VRE: Vancomycin-resistant enterococci.

https://pubmed.ncbi.nlm.nih.gov/12783569/

Vancomycin resistance

Enterococcus spp.



https://commons.wikimedia.org/wiki/File:Vancomycin_resistance.svg

Vancomycin resistance

E. faecium & *E. faecalis*
can acquire *vanA* or *vanB*.

http://cmr.asm.org/content/13/4/686.full.pdf

Vancomycin resistance

E. gallinarum & *E. casseliflavus*
have chromosomal *vanC*.

These species should be considered intrinsically resistant to vancomycin, regardless of *in vitro* susceptibility test results.

Vancomycin intrinsic resistance

Cocci:

- *Pediococcus*
- *Leuconostoc*
- *Weissellla*
- *Enterococcus gallinarum*
- *E. casseliflavus*

Bacilli:

- *Erysipelothrix*
- *Lactobacillus* (some species)

Vancomycin resistance

Staphylococcus

Gram positive organisms (other than enterococci) have acquired *vanA*, but this phenomenon is currently exceedingly rare.

Vancomycin resistant *S. aureus* = VRSA

Vancomycin resistance

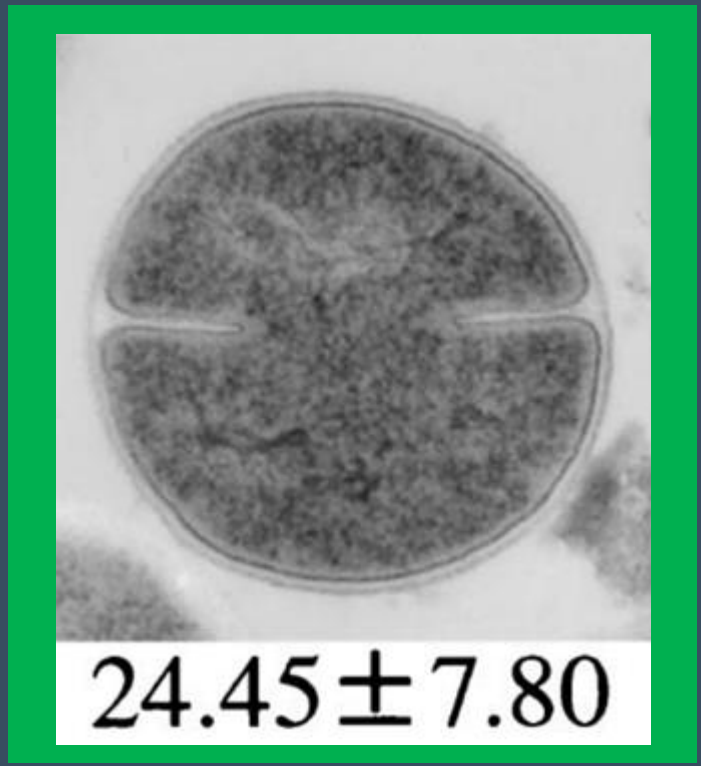
Staphylococcus

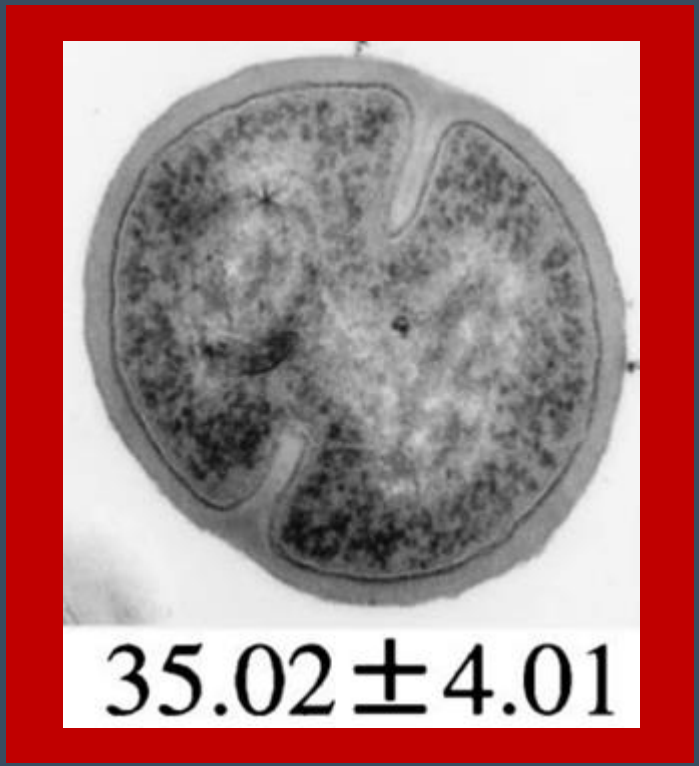
Vancomycin non-susceptibility typically occurs due to “creep,” which appears to correlate with cell wall thickness.

Uncommonly, vancomycin intermediate *S. aureus* (VISA) can be identified.

Vancomycin resistance

Staphylococcus





J Clin Microbiol. 2003 Jan;41(1):5-14.

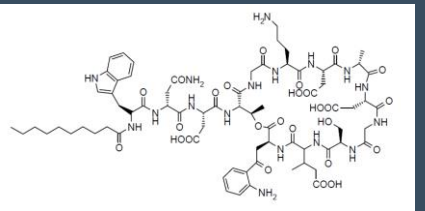
Daptomycin

MOA: Daptomycin aggregates in the cell membrane and causes defects leading to cell death.

Target bacteria: gram-positive bacteria

MOR: Resistance is not well understood, but resistance is thought to be linked to modification of the phospholipid composition of the cell membrane.

Daptomycin is bound by the surfactant in the lung, so it should not be reported on isolates obtained in respiratory specimens.



https://pubmed.ncbi.nlm.nih.gov/22661688/
https://pubmed.ncbi.nlm.nih.gov/12783569/

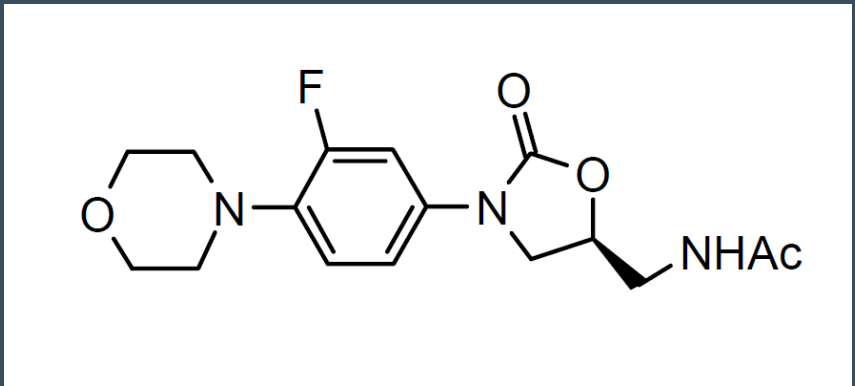
Linezolid

MOA:
Oxazolidinones bind to the 50S ribosomal subunit to prevent initiating translation of mRNA.

“This mode of action differs from that of existing protein synthesis inhibitors such as chloramphenicol, macrolides, lincosamides and tetracyclines, which allow mRNA translation to begin but then inhibit peptide elongation.”

Target bacteria: gram-positive bacteria

MOR:
Resistance to linezolid is gained by point mutation(s) in the ribosomal RNA (rRNA).



https://pubmed.ncbi.nlm.nih.gov/27999068/
https://pubmed.ncbi.nlm.nih.gov/12783569/

Macrolide / Lincosamide / Streptogramin

(erythromycin, clarithromycin, azithromycin, clindamycin)

MOA:
Translation is abandoned early. Peptidyl-tRNAs are released from the ribosome before translation can be completed.

Target: Mostly gram-positive bacteria

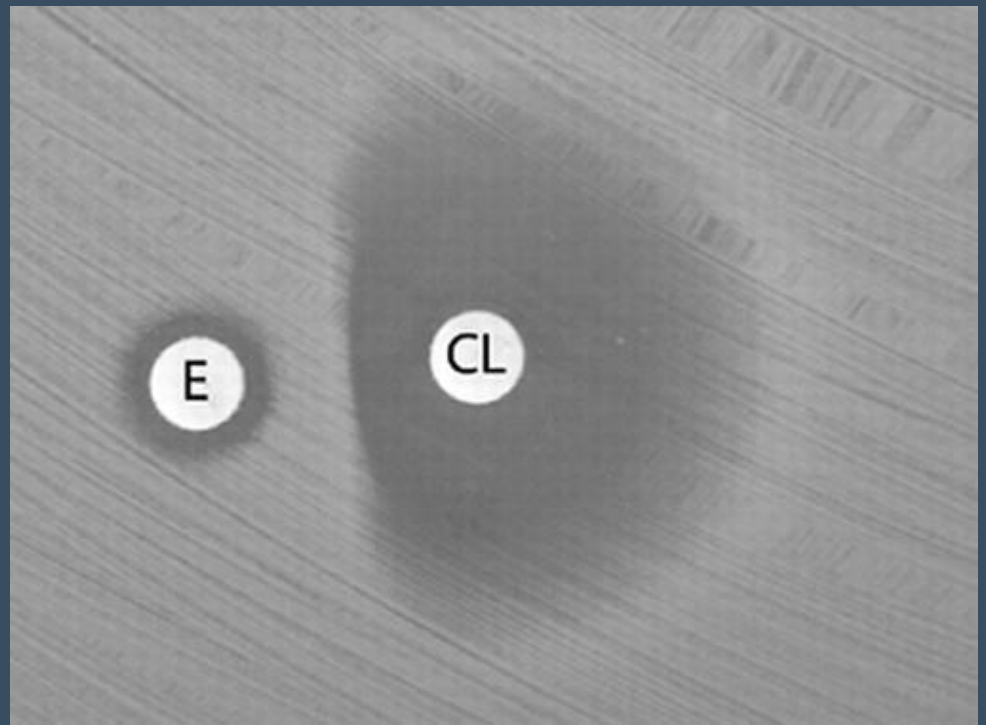
https://pubmed.ncbi.nlm.nih.gov/12860123/

Macrolide

(erythromycin, clarithromycin, azithromycin)

MOR:

1) *erm* (erythromycin ribosomal methylase)



http://aac.asm.org/content/49/3/1222/F1.large.jpg
https://pubmed.ncbi.nlm.nih.gov/11797175/

Macrolide

(erythromycin, clarithromycin, azithromycin)

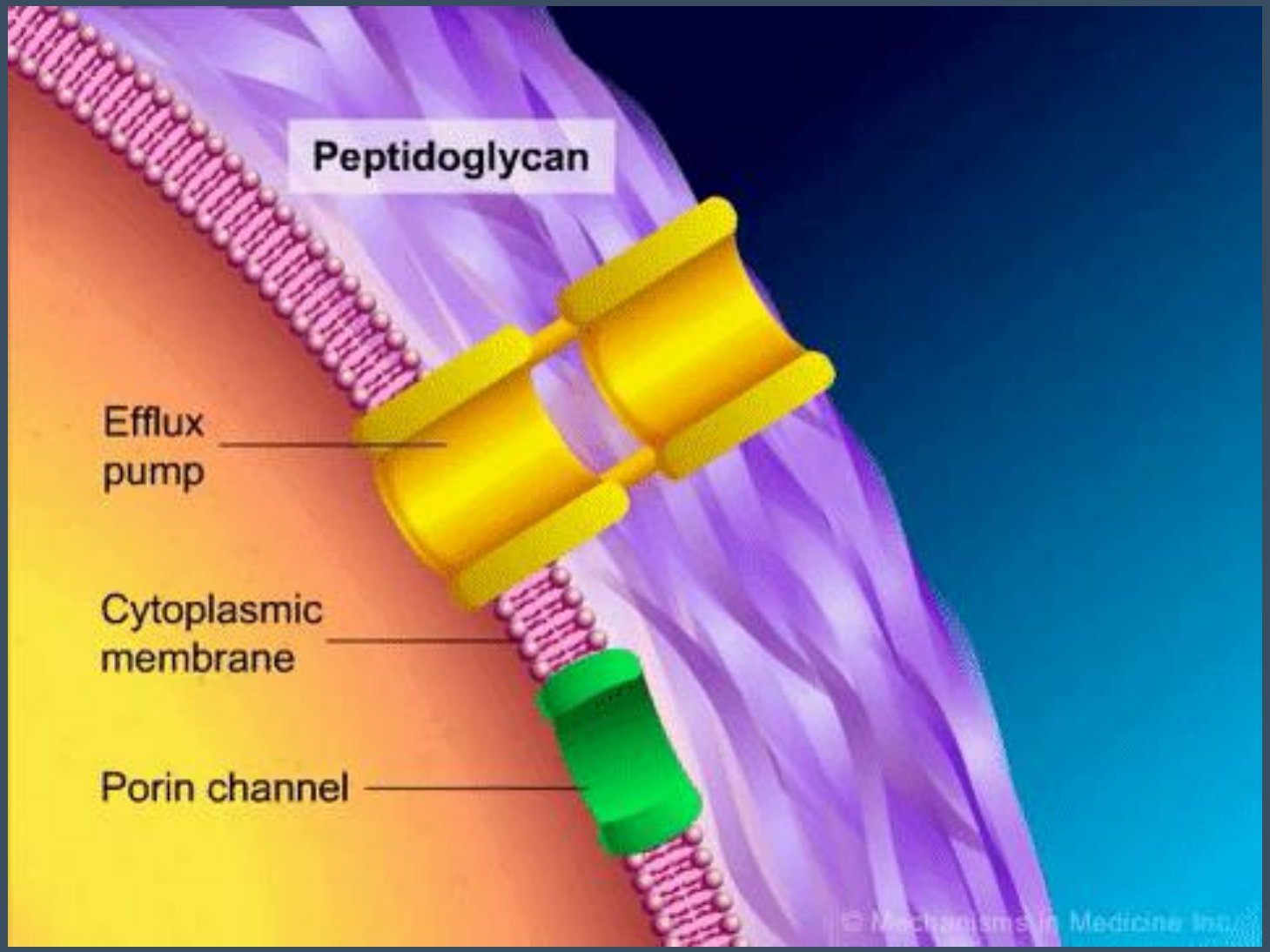
MOR:

1) *erm* (erythromycin ribosomal methylase)
2) *mef* (macrolide efflux), which invokes macrolide resistance without clindamycin resistance.

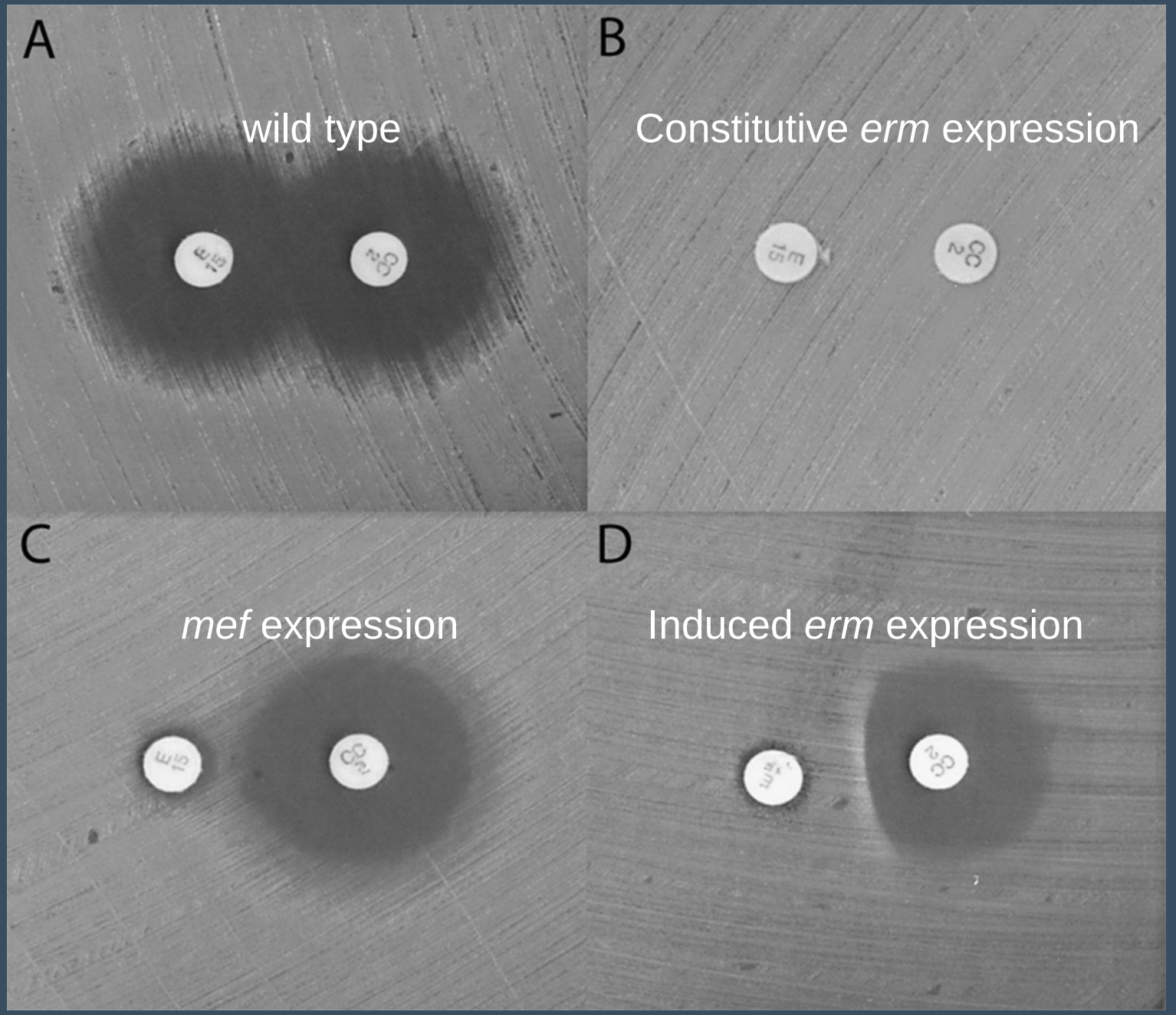
https://pubmed.ncbi.nlm.nih.gov/12860123/
https://www.sciencedirect.com/science/article/pii/S1201971216000369

Macrolide

mef (macrolide efflux)



https://www.youtube.com/watch?v=oC21vLFtsjo&



http://jcm.asm.org/content/51/12/4196/F1.large.jpg

Macrolide

(erythromycin, clarithromycin, azithromycin)

MOR:

1) *erm* (erythromycin ribosomal methylase)
2) *mef* (macrolide efflux), which invokes macrolide resistance without clindamycin resistance.
3) Drug modification (i.e. esterases & phosphotransferases)

https://pubmed.ncbi.nlm.nih.gov/12860123/
https://www.sciencedirect.com/science/article/pii/S1201971216000369

Rifampin

MOA:
Inhibits transcription

Target bacteria: Mostly gram-positive bacteria

MOR:
Resistance can occur due to mutations in *rpoB*, which encodes for RNA polymerase.

https://pubmed.ncbi.nlm.nih.gov/6356275/

Fluoroquinolones

(ciprofloxacin, levofloxacin, moxifloxacin)

MOA:

- DNA gyrase (topoisomerase II) in gram-negative bacteria
- Topoisomerase IV in gram-positive bacteria

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC122823/

Fluoroquinolones

(ciprofloxacin, levofloxacin, moxifloxacin)

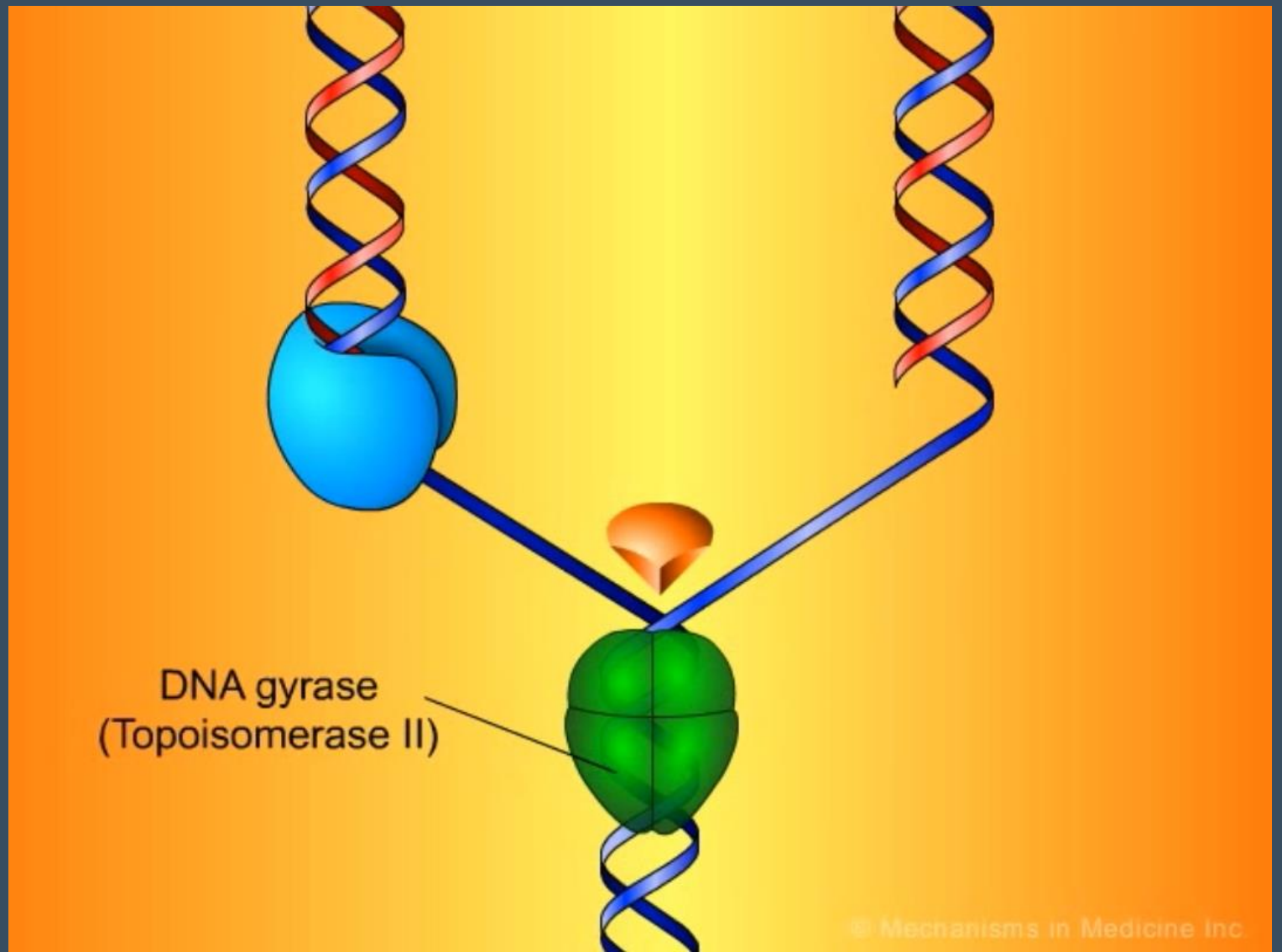
MOR:

- Efflux and porins
- Spontaneous mutation(s) in topoisomerases (e.g. *gyrA*), which causes target modification
- Plasmid-mediated acquisition of quinolone resistance genes (*qnr*), which protect topoisomerase from quinolone activity.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC122823/

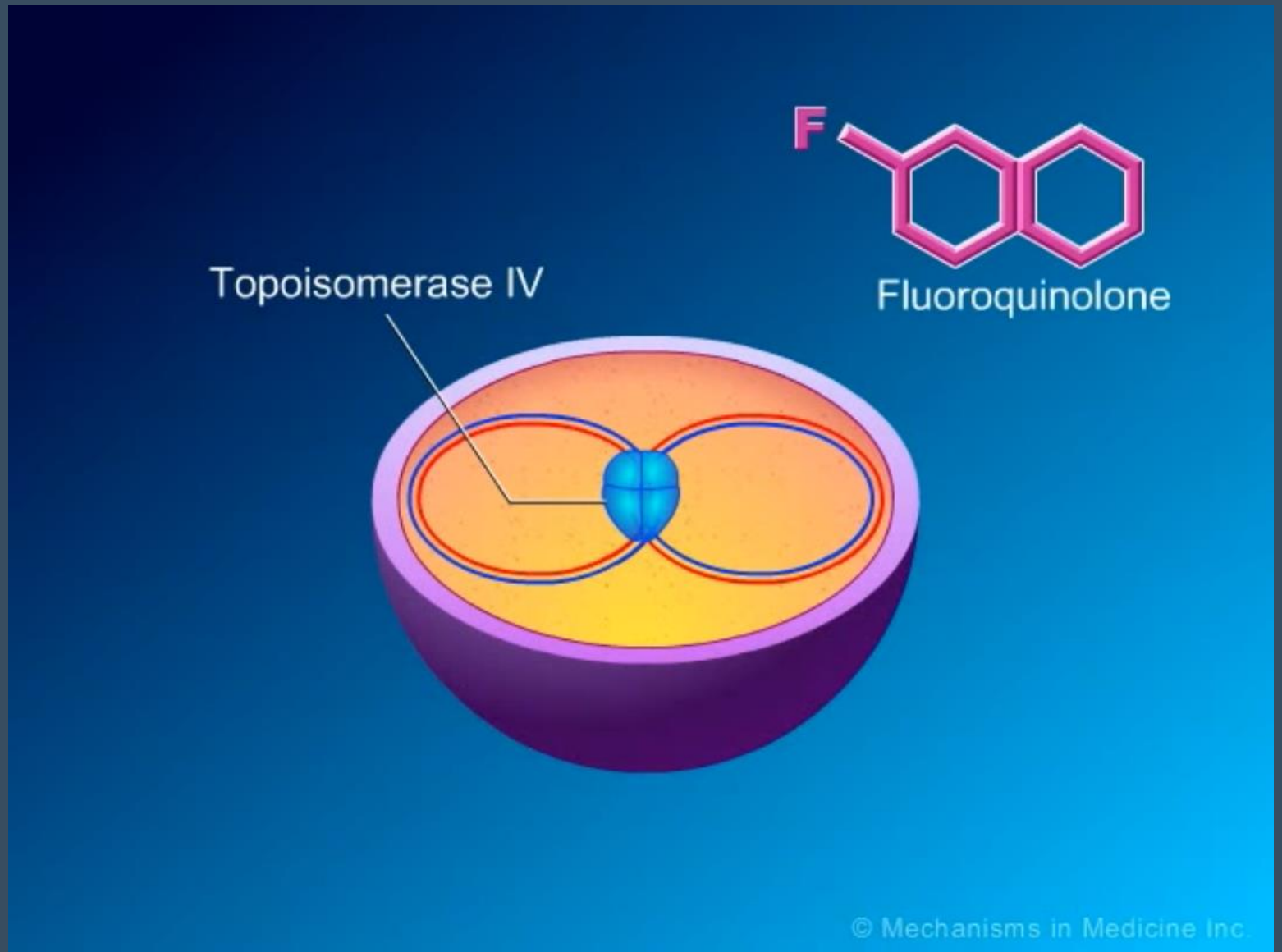
Fluoroquinolones

Primary Gram negative target



Fluoroquinolones

Primary Gram positive target



Aminoglycosides

(gentamicin, tobramycin, amikacin)

MOA: foster translational errors

Target bacteria: Mostly gram-negative bacteria

MOR:

1) Decreased permeability & increased efflux
2) Target modification (i.e. ribosome)
3) Enzymatic inactivation of the drug through phosphorylation, acetylation, or adenylation.

https://pubmed.ncbi.nlm.nih.gov/17447006/

Tetracyclines

(doxycycline, minocycline, tigecycline, eravacycline)

MOA: interferes with translation

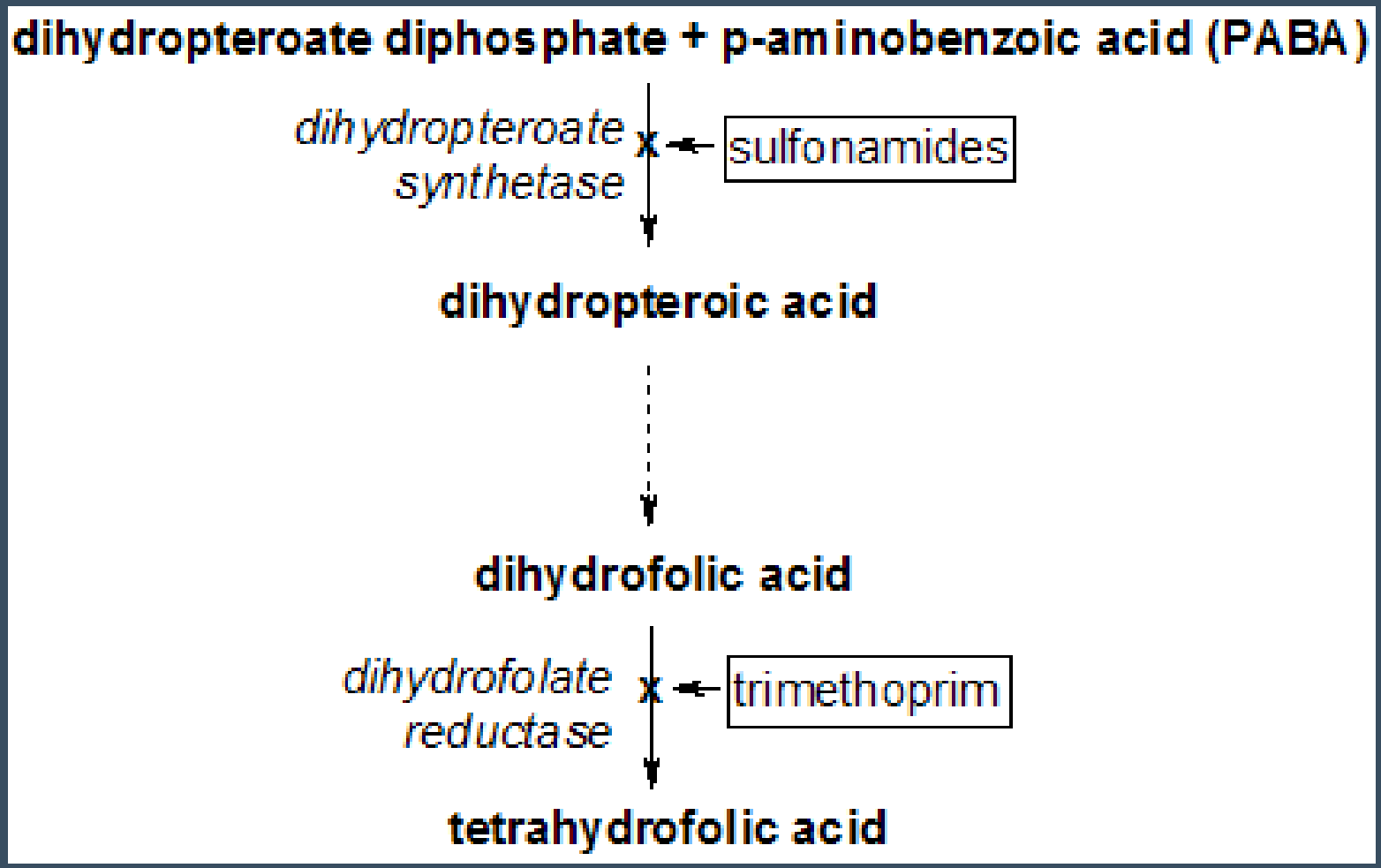
Target bacteria: Gram-negative and positive bacteria

MOR:

1) Increased efflux
2) Target modification (i.e. ribosome)
3) Enzymatic inactivation of the drug

https://pubmed.ncbi.nlm.nih.gov/1423217/

Trimethoprim / sulfamethoxazole



Trimethoprim / sulfamethoxazole

MOA: Folate metabolism interference

Target bacteria: gram-negative & positive bacteria

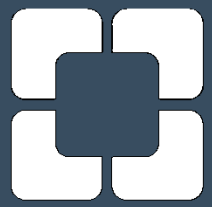
MOR:
Multiple pathways to resistance including porins, efflux, overproduction of the competitively inhibited enzymes, mutations in native *dhfr* or *dhps*, or acquisition of low-affinity DHFR or DHPS enzymes.

Links & References

http://www.mechanismsinmedicine.com/site/index/animation/infectious-diseases

Mandell's Infectious Diseases, 8th ed. Elsevier Saunders. Chapter 18.

Manual of Clinical Microbiology, 12th ed. ASM Press. Chapters 70-79.



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