Mechanisms of Antibiotic Resistance in Bacteria

Mechanisms of ATB Resistance

NATURAL/INTRINSIC

- Intrinsic resistance is **when a bacterial species is naturally resistant to a certain antibiotic or family of antibiotics, without the need for mutation or gain of further genes**. This means that these antibiotics can never be used to treat infections caused by that species of bacteria. Intrinsic resistance usually **mediated by the bacterial outer membrane** but also by antibiotic efflux.

MAIN ACQUIRED RESISTANCE MECHANISMS

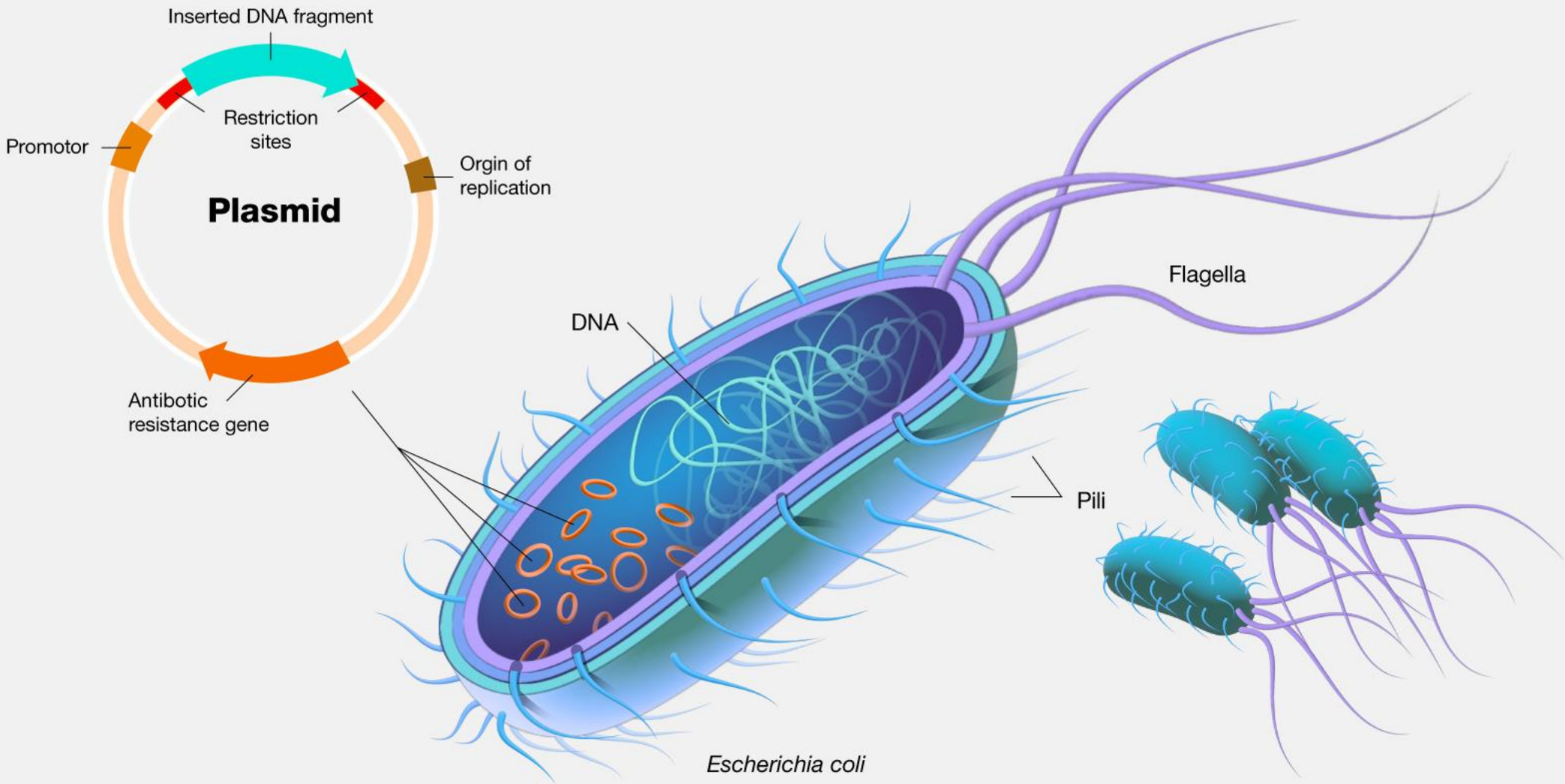
- 1. ENZYMATIC INACTIVATION OF ANTIBIOTICS
- 2. ANTIBIOTIC MODIFICATION
- 3. ALTERED TARGET SITE
- 4. MECHANISMS OF ANTIBIOTIC EFFLUX
- 5. DECREASED PERMEABILTY OF BACTERIAL MEMBRANES

ACQUIRED MECHANISMS OF RESISTANCE

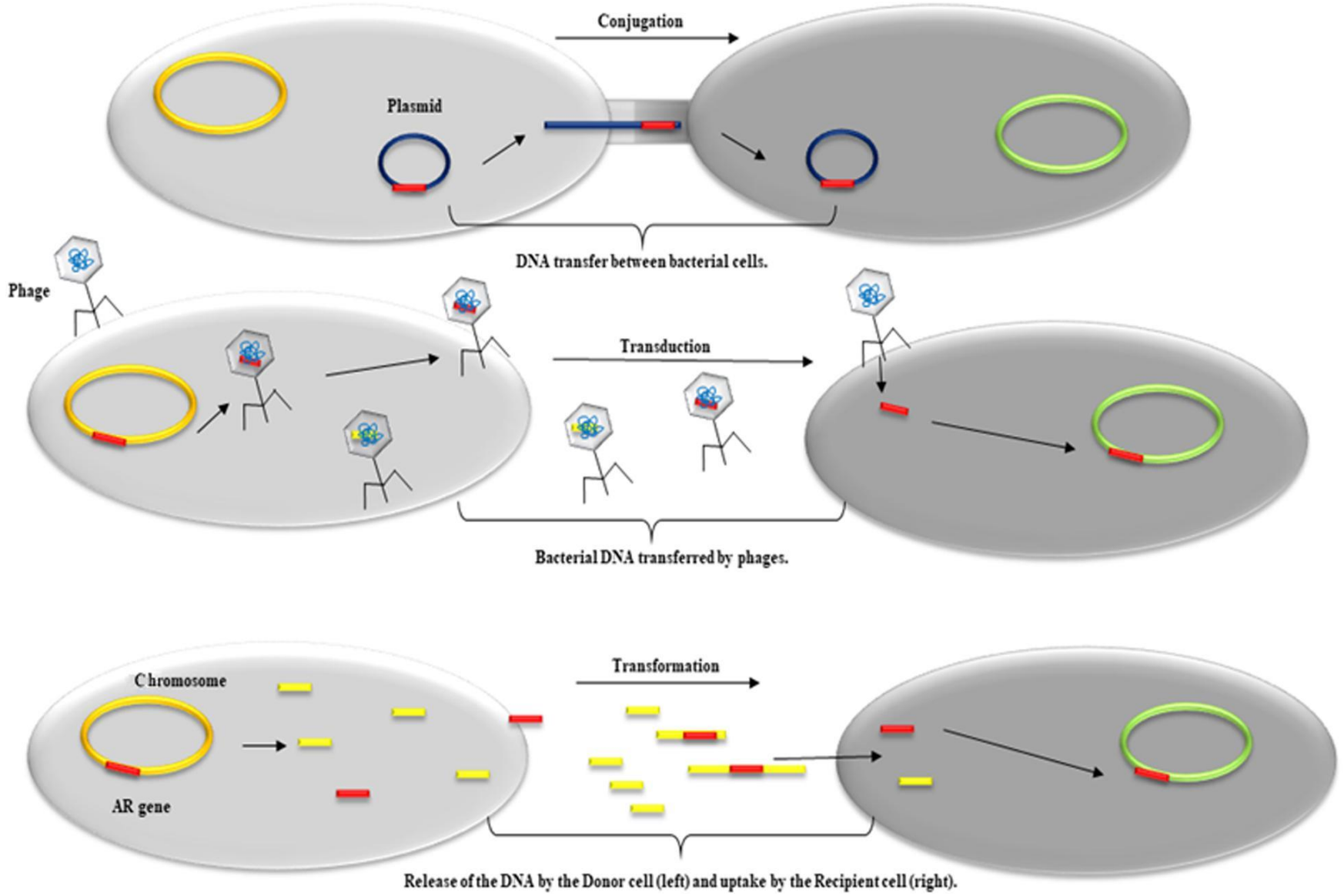
- Microbial genetic variability through variety of mechanisms (**point mutations**, **rearrangement of large DNA segments – inversions, duplications, insertions, deletion or transpositions** ususally generated by integrons, transposons, insertion sequences, acquisitions of foreign DNA carried by plasmids, bacteriophages, naked DNA sequences, transposable genetic elements) is **essential for microbial evolution to occur**
- **Antimicrobials (ATB) exert strong selective pressures on microbial populations**
- **ATB resistence genes** were **present in the era before ATB** therapy was available – probably **originated from antibiotic-producing bacteria**
- **These resistant bacterial populations spread** ATB **resistance vertically** to subsequent generations and horizontally **to susceptible strains of related bacteria**, even to different species or genera

Plasmids

- **Extrachromosomal elements** – agents of genetic exchange and resistance-gene dissemination
- **Autonomously replicating – double-stranded DNA circular,** covalently closed **(10-400 kbp)**
- Extremely **common in bacteria, multiple copies** of specific plasmid **or multiple different plasmid** – in a bacterial cell
- ***tra* genes needed for transfer** make conjugative plasmids larger
- **small plasmids** using conjugation **apparatus from coresident conjugative plasmids or conjugative transposons**


Inserted DNA fragment
Restriction sites
Promotor
Orgin of replication
Plasmid
Antibotic resistance gene
DNA
Flagella
Pili
Escherichia coli

Plasmids



Ref: https://www.frontiersin.org/articles/10.3389/fmicb.2020.00761/full

Transposable genetic elements (transposons - Tn, isertion sequences – IS)

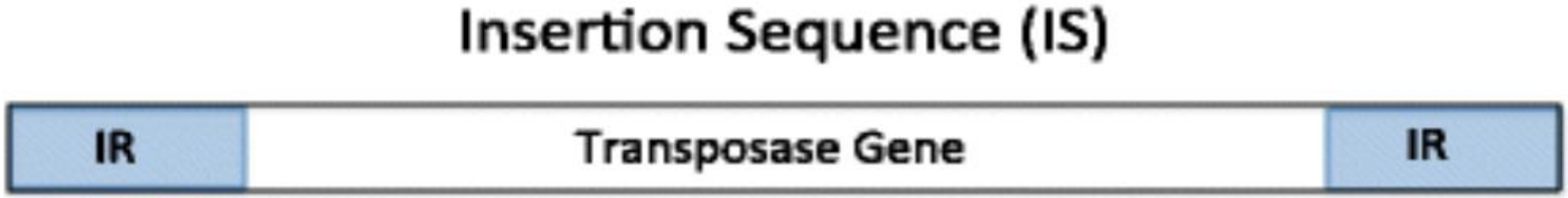
- Transposons translocates a unit **from one area of the bacterial chromosome to another** or **between the chromosome and plasmid or bacteriophage** DNA
- **Transposable genetic elements** posses **specialized system of recombination**

(recA independent recombination system) **transposase – occurs between nonhomologous sequences of DNA, results in wholesale modifications of large sequences of DNA**

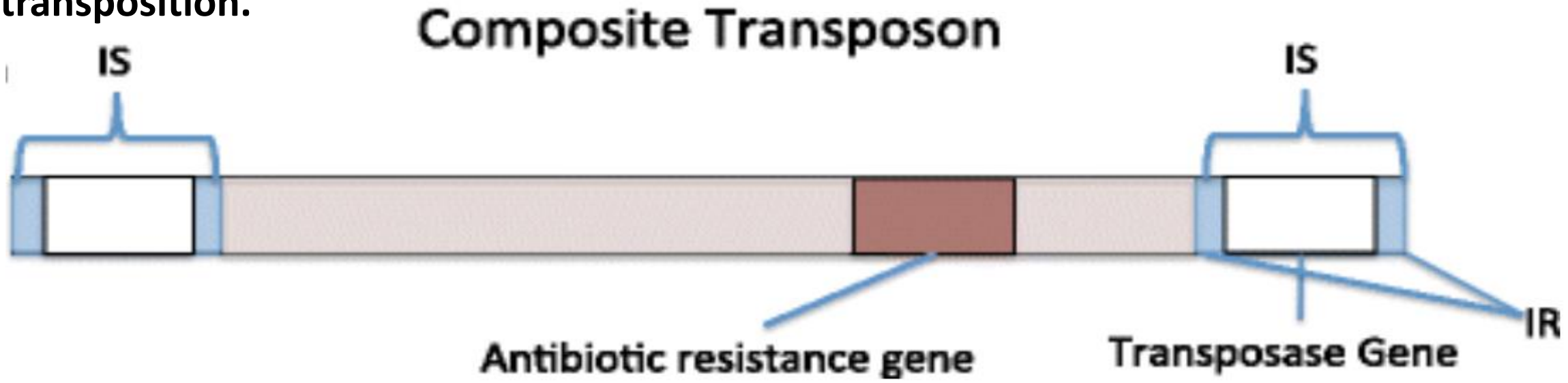
- **Tn, IS** (except conjugative transposons) **– incapable of autonomous self-replication and must exist on a replicon** (chromosome, bacteriophage, plasmid)

- **Transposition is a continuous, ongoing proces** in bacterial population (e.g. spread of tetracyclin-resistance Tn among. *N. gonorrhoeae, Mycoplasma hominis* and *Ureoplasma urealyticum; vanA gene* mediated in enterococci by a composite transposon*)*
- **Transposons – essential** in the **evolution of resistance plasmids**

Structure of transposable genetic elements (transposons - Tn, isertion sequences – IS)



The insertion sequence (IS) is the smallest transposon present in bacterial chromosomes and plasmids. It is composed of two inverted repeats (IR) flanking genes necessary for transposition.

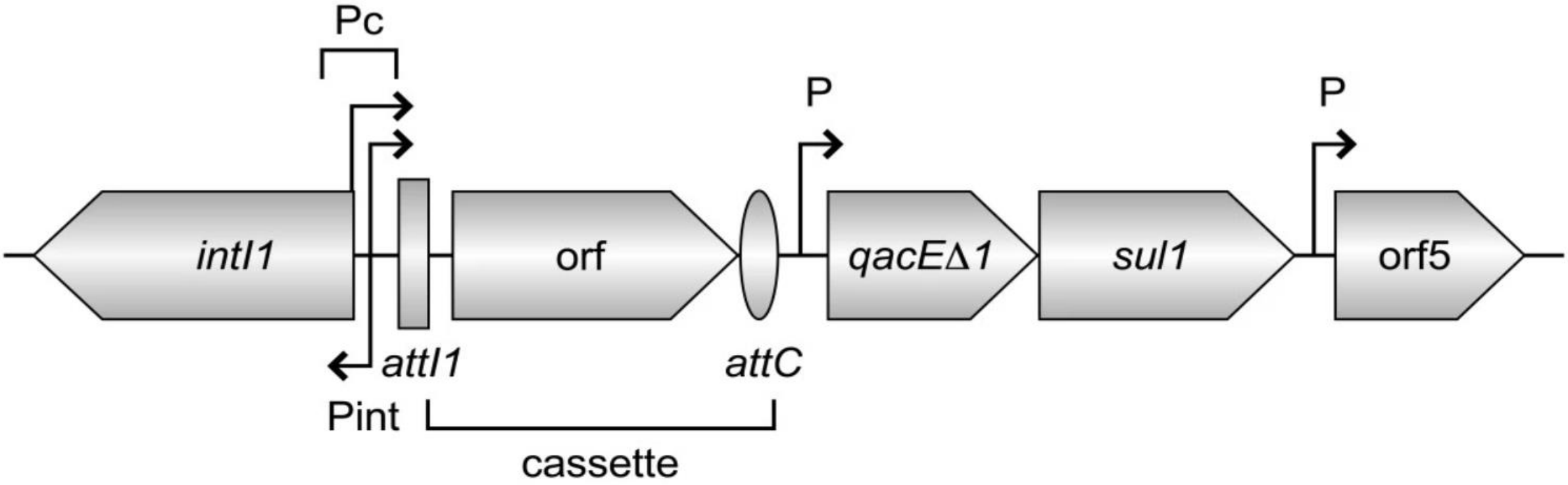


The composite transposon is composed of two IS elements flanking a central protein coding DNA region. This central region often contains genes for antibiotics resistance

DNA Integration Elements

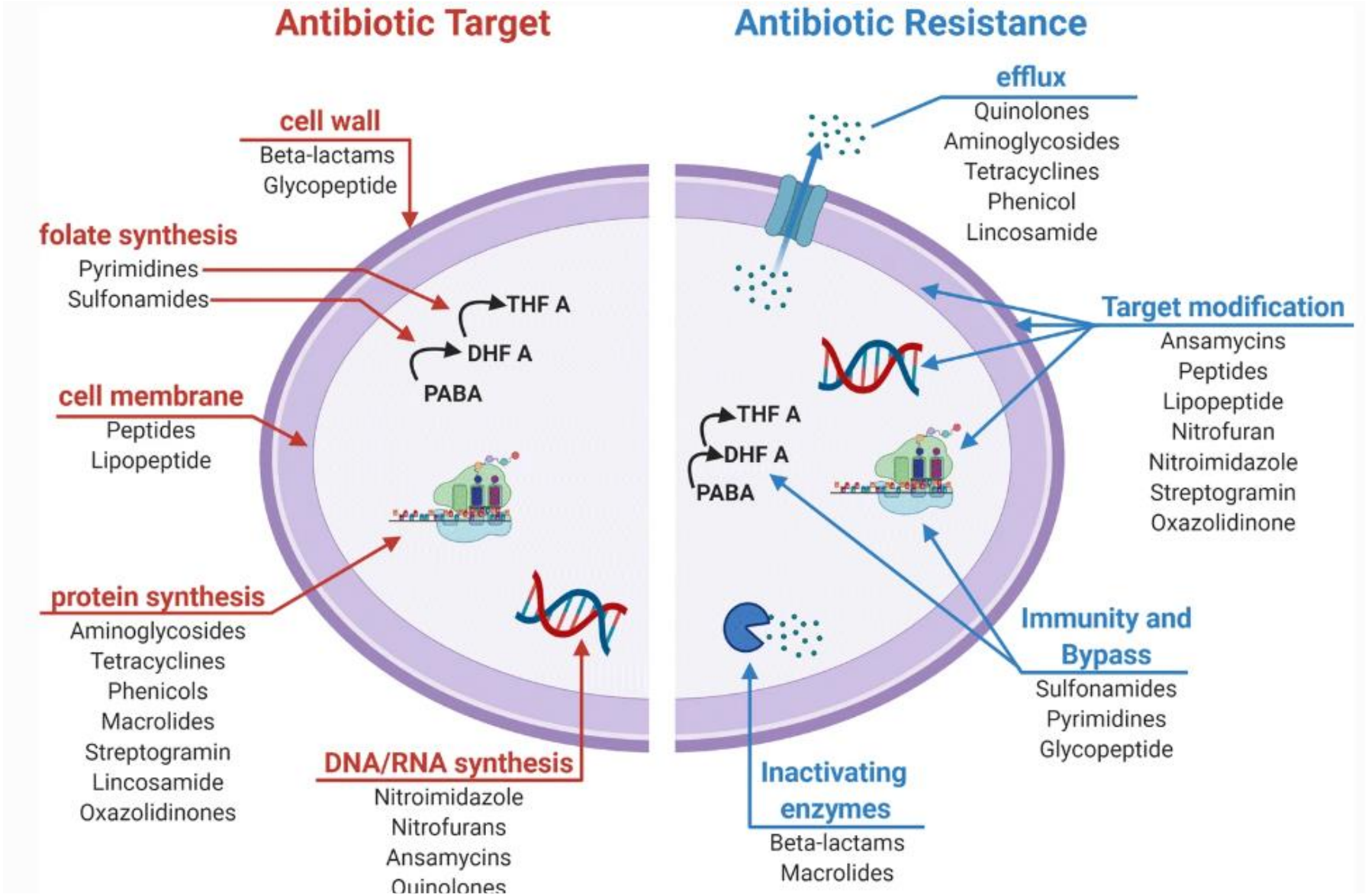
- **Integrons -** ATB resistance genes are often closely linked, may exist in tandem along bacterial chromosome or plasmid
- **Principal role** – provide a convenient **insertion site for ATB resistence genes from foreign DNA sources**
- **Integrons -** often exist **near promoter site**
- **recombinational „hot spots“ for site-specific recombination events between large nonhomologous sequences**
- Own **integrase function (integrated specialized attachment and integration site** – from 57 – 141 bp**)** – mediated ATB resistance genes from **mobile gene cassettes**
- **Frequency of transcription of integrated cassetes of ATB resistance genes depends on proximity to the promoter at the 5´** upstream end of the region

General structure of class 1 integrons



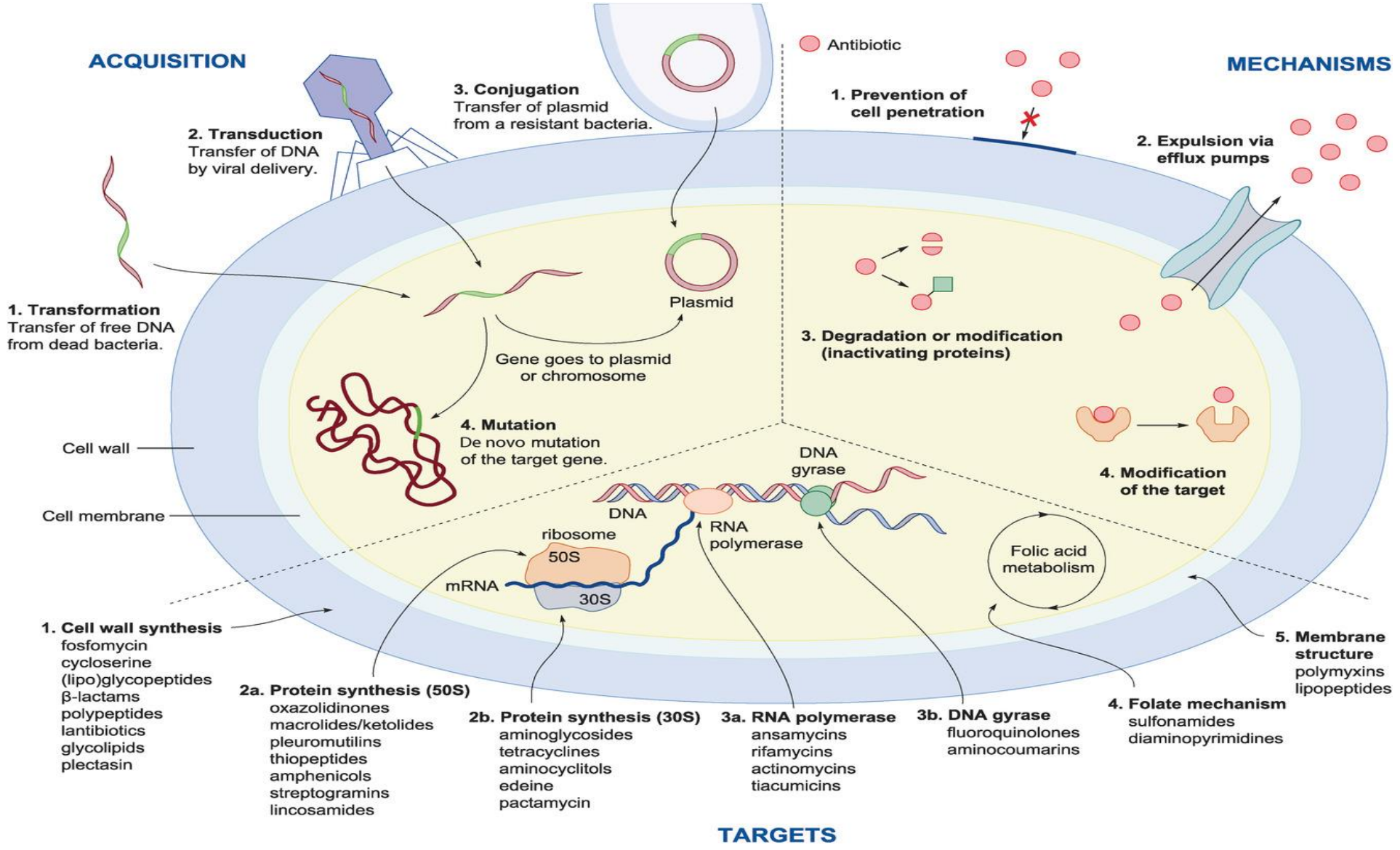
General structure of class 1 integrons. Cassettes are inserted in the variable region of integrons by a site-specific recombination mechanism. The *attI1* and *attC* sites are shown by a vertical rectangle and oval, respectively, and promoters are denoted by Pint, Pc and P. Integrated cassettes are composed of a gene and an *attC* recombination site. Genes are as follows: *intI1*, integrase gene; *qacEΔ1*, antiseptic resistance gene; *sul1*, sulphonamide resistance gene; orf5, gene of unknown function.

Diagram of Antibiotic Target and Resistance Mechanisms



Ref: Cardoso P. Molecular engineering of antimicrobial peptides: microbial targets, peptide motifs and translation opportunities, 2021

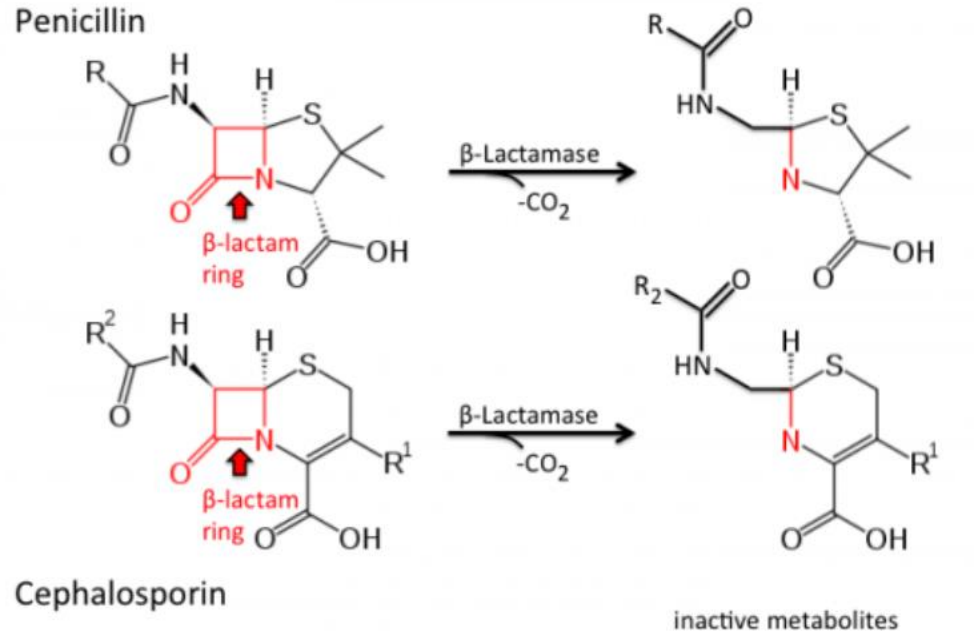
Diagram of Antibiotic Target and Resistance Mechanisms



Ref: Angew Chem Int Ed, Volume: 55, Issue: 23, Pages: 6600-6626, First published: 22 March 2016, DOI: (10.1002/anie.201506818)

1. ENZYMATIC INACTIVATION OF ANTIBIOTICS

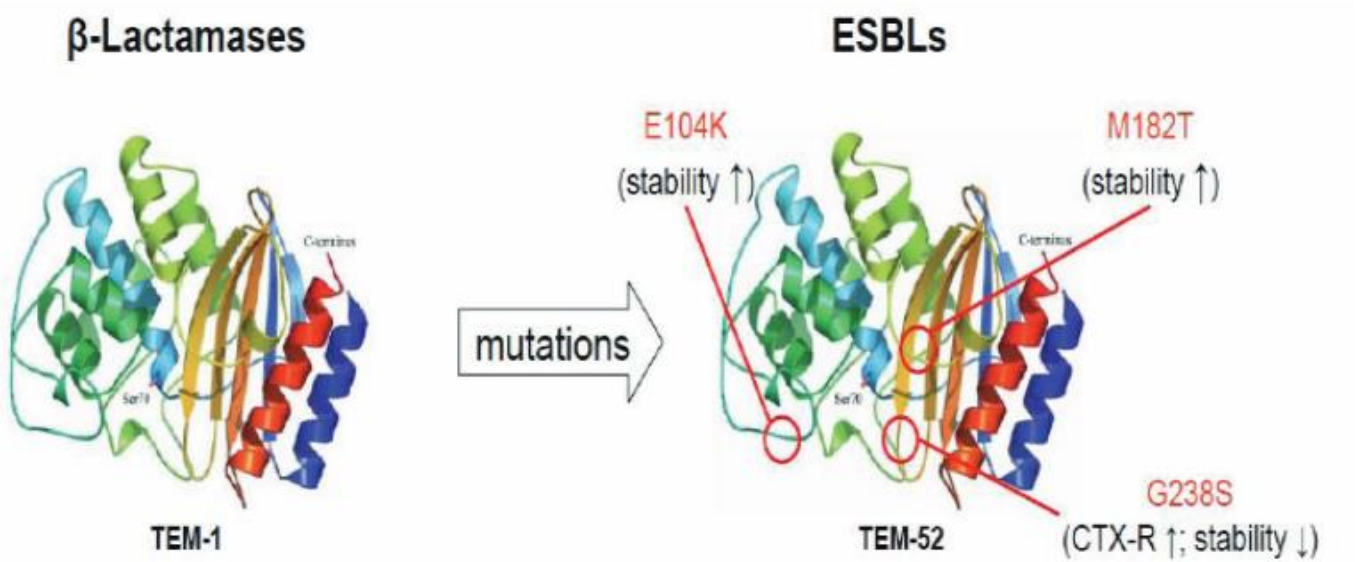
- **Betalactamases**
- a) **resistance to betalactams primarily occurs through betalactamases** – splitting the amide bond of the betalacm ring
- b) Extended-Spectrum Betalactamases
- c) Carbapenemases



Ref: https://tmedweb.tulane.edu/pharmwiki/doku.php/betalactam_pharm

- Most likely **co-evolved wit bacteria as mechanisms against natural antibiotics**
- Selective presure exerted by **widespread use of ATB** have **acclerated** their **development** and **spread**
- Can be **located** either on **chromosomes** or transferable genes locate on **plasmids** or **transposons, integrons** (can facilitate MDR resistance).
- **Classification** (Ambler – according to their **amino acid structure** A-D or Bush-Jacoby-Medeiros according to **functional substrate profile and susceptibility to inhibitors**)

Betalactamases structure



Spectrum:

- penicillins
- 1st generation cephalosporins
- 2nd generation cephalosporins

Extended Spectrum:

- penicillins
- 1st generation cephalosporins
- 2nd generation cephalosporins
- **3rd generation cephalosporins (e.g. cefotaxime)**
- **monobactams (e.g. aztreonam)**

Susceptible to cephamycins (cefotetan, cefoxitin), carbapenems (e.g. imipenem), and inhibitors

Ref: Ghafourian S., Extended Spectrum Beta-lactamases, Curr. Issues Mol. Biol. (2015) 17: 11-22.

Ambler classification of betalactamases (classes A – D)

	Active site	Enzyme Type	Substrates	Examples
A	Serine	**Penicilinases** Broad-spectrum	benzylpenicillin,aminopenicillinis,carboxypenicillins,ureidopenicillins,narow-spectrum cephalosporins	PC1 in S. aureus,TEM-1,SHV-1 in *E.coli,K.pneumonia* and other Gramneg.bacteria
		Extended-spectrum (ESBL)	Substrates of broad-spectrum plus oxyimino-betalactams (cefotaxime,ceftazidime,ceftriaxone), aztreonam	e.g. *E.coli* (derived from TEM,SHV,CTX-M), *P. aeruginosa* IBC-2
		Carbapenemases	Substrates of ESBL plus cephamycins (e.g. cefuroxime) and carbapenems	e.g. *K. pneumonaie* KPC-1, 2, 3
B	Metallo-ß-lactamases (Zn^{2+})	**Carbapenemases**	Substrates of ESBL plus cephamycins (e.g. cefuroxime) and carbapenems	e.g. VIM, IMP, SIM lineages in *P. aeruginosa*
C	Serine	**Cephalosporinases**	Substrates of ESBL plus cephamycins (e.g. cefuroxime)	e.g. AmpC-type enzymes in Enterobacteriaceae, *Acinetobacter*
D	Serine	**Oxacillinases**		
		Broad-spectrum	aminopenicillins, ureidopenicillins, oxacillin, some narow-spectrum cephalosporins	e.g. OXA family in *P.aeruginosa*
		Extended-spectrum	Spectrum of broad-spectrum plus oxyiminobetalactams (cefotaxime,ceftazidime) and monobactam (aztreonam)	e.g. OXA-derived in *P.aeruginosa*
				e.g. OXA-derived in *Acinetobacter*

Note: Classification A – C is most relevant for medical students to know

Betalactamases

- One of the first betalactamase described was **penicillinase in *S. aureus*** (could be used and inhibitor b-lactamase–b-lactamase inhibitor complex binding the enzyme). **High levels of resistance to penicillin (80-95%)** are standard **for community strains** in almost all countries now.
- **In Gramnegative bacteria** – rise in **ampicilin** in 1960s emergence of TEM-1 (Greek patient Temoneira), plasmid encoded, was later disseminated as **family TEM betalactamases in *P. aeruginosa*, Enterobacteriaceae, *H. influenzae*, *N. gonorrhoeae*.**
- **Similarly chromosomaly- and plasmid-mediated SHV-type betalactamases** (molecular structure similar to TEM) became **widely prevalent among *E. coli* and *K. pneumoniae*.**
- **Third-generation cephalosporin were stable to them but ESBL are capable of hydrolyzing broad-spectrum cephalosporins and monobactam (aztreonam).**
- In addition, **increasing reports of carbapenemases** have arisen **concern** about currently **limited number of antimicrobial arsenal against infection caused by MDR Gramnegative bacteria.**

ESBL

- **TEM-derived: the most common in Gramneg. bacteria**, **hydrolyze penicillin and narow-spectrum cephalosporins** in Enterobactariaceae, *N. gonorrhoeae*, *H. influenzae*. **ESBL** is obtained **through changes** in a **single or few amino acids** that **alter configuration of the enzyme active site** more **accesible to** oxyimino-side chain of **3rd cephalosporines** (more than 160 enzymes, primarily in *E. coli*, *K. pneumoniae*, majority TEM-derived ESBL remain susceptible to clavulanic acid)
- **SHV-derived:** SHV-1 similar to that of TEM-1, primarily in *K. pneumoniae*
- **CTX-M-derived:** probably acquired by plasmid from the chromosomal AmpC enyzymes of low pathogenic Granegative rod *Kluyvera*, hydrolyze cefotaxime, ceftriaxone, ceftazidime, most prevalent in Europe and South America
- **OXA-derived ESBL:** hydrolyze oxacillin and its derivate, described mainly in *P. aeruginosa*
- **AmpC enzymes: primarily chromosomal enzymes that confer resistence to penicillins, narow-spectrum cephalosporins, oxyimino-betalactams, cephamycins (e.g. cefoxitin), not susceptible to inhibitor of betalactamases** (detected in some clinical strains of e.g. *Enterobacter, Citrobacter, Serratia*)

***SIGNIFICANT NOTE**: if we do not know what type of broad-spectrum beta-lactamase the bacterium produces, we cannot use beta-lactam with a beta-lactamase inhibitor in treatment !!!*

Carbapenemases

- **confer resistance the largest ATB spectrum**
- **KPC the most important carbapenemases** (initially reported K. pneumoniae)
- **Resistant to inhibitors** - clavulanic acid, tazobactam, sulbactam
- Confer **resistance to all betalactams and monobactam**
- **chromosomaly encoded metallo-betalactamases (e.g. VIM, IMP…) primarily found in environmental isolates of *Aeromonas, Chryseobacterium ans Stenotrophomonas***
- **typically transmitted by mobile genetic elements (MGE)** inserted into **integrons – have** spread through *P. aeruginosa, Acinetobacter*, other Nonfermenters and enteric bacterial pathogens
- class D carbapenemases (OXA – 23,24,40,58) primarily found in Acinetobacter

2. ANTIBIOTIC MODIFICATION

- **Aminoglycoside (AMG) Resistance-Modifying Enzymes** (more than 30 enzymes)
- AMG resistance is **the most commonly caused by modifying enzymes**
- encoded **on plasmid** or **chromosome**
- **3 general reactions: N-acetylation, O-nucleotidylation, O-phosphorylation** (attack specific amino or hydroxyl group)
- **Nomenclature: where the modification occurs**
- Modification of ATB during transport across the cytoplasmic membrane
- **APH (3´) and APH (3´´)** distributed widely among **Grampositive** and **Gramnegative** bacteria
- **AAC(6´)APH(2´**) is a **bifuntional enzyme (acetylation/phosporylation)** widespread in **staphylococci** and **enterococci**), residing in a common transpozon Tn4001

Representative aminoglycosides and modification sites by AAC, ANT, and APH enzymes

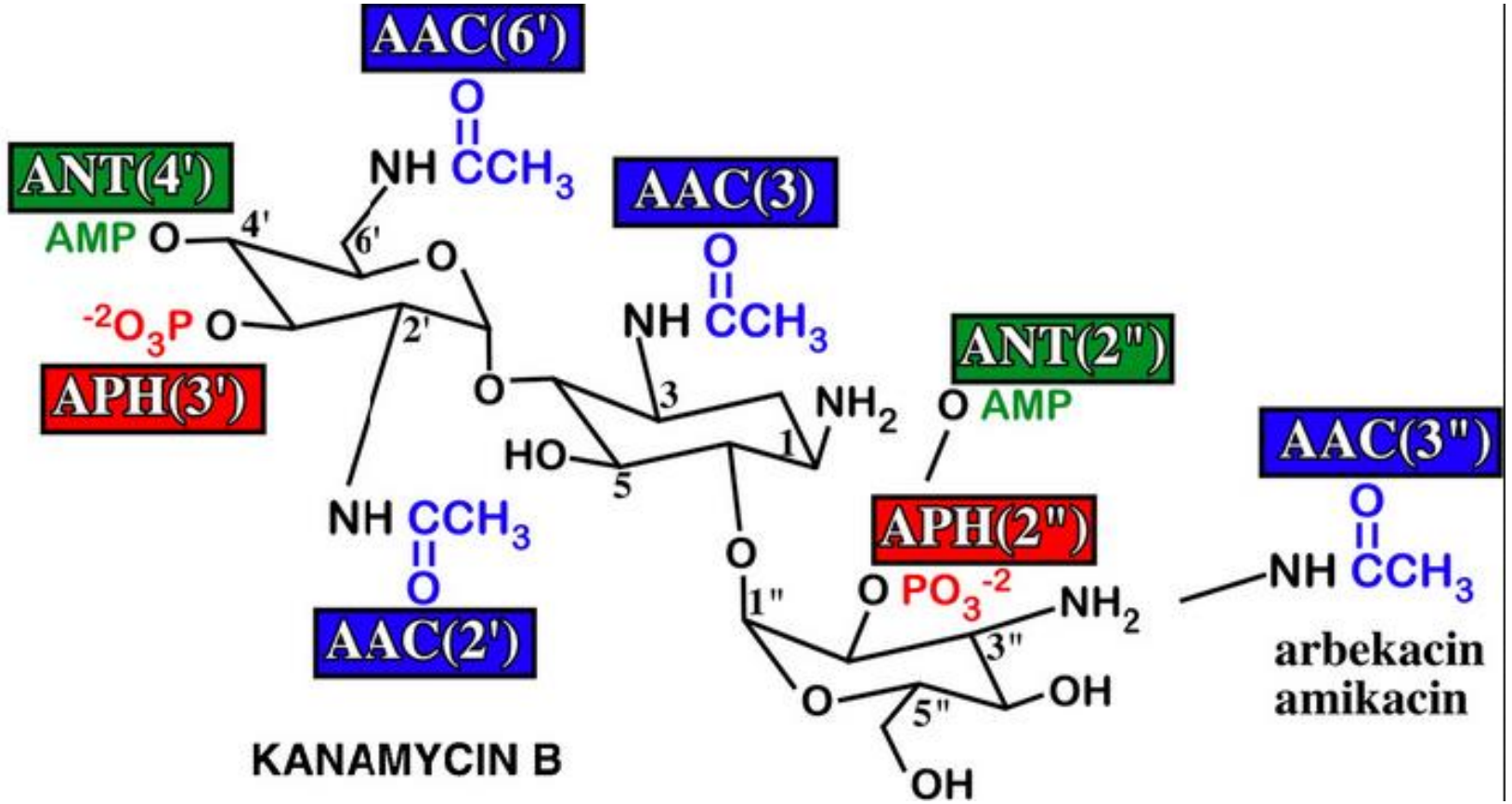


Fig. An example of each kind of modification is shown on one of the substrates. The square and oval on positions 2′ and 6″ in paromomycin I indicate that although this molecule is preferentially acetylated at the position 1, 1,2′-di-N-acetylparomomycin and 1,6″-di-N-acetylparomomycin are also found as products of the enzymatic reaction (Sunada et al., 1999). AAC(3)-X can catalyze acetylation at the 3″-amino group in arbekacin and amikacin (Hotta et al., 1998). (Ref: https://www.sciencedirect.com/science/article/pii/S1368764610000385#fig0005)

Mechanisms of Chloramphenicol Resistance

- **Chloramphenicol acetyltransferase (plasmid- or chromosome-borne) and acetylation** is primary mechanism of the resistance in **Grampositive** and **Gramnegative bacteria**

Macrolide-, Linkosamide-, Streptogramin- Inactivating Enzymes

- **Erythromycin esterases – hydrolyse the lactone ring** and limit **erythromycin and other macrolides** use to treat **Gramnegative** bacterial pathogens

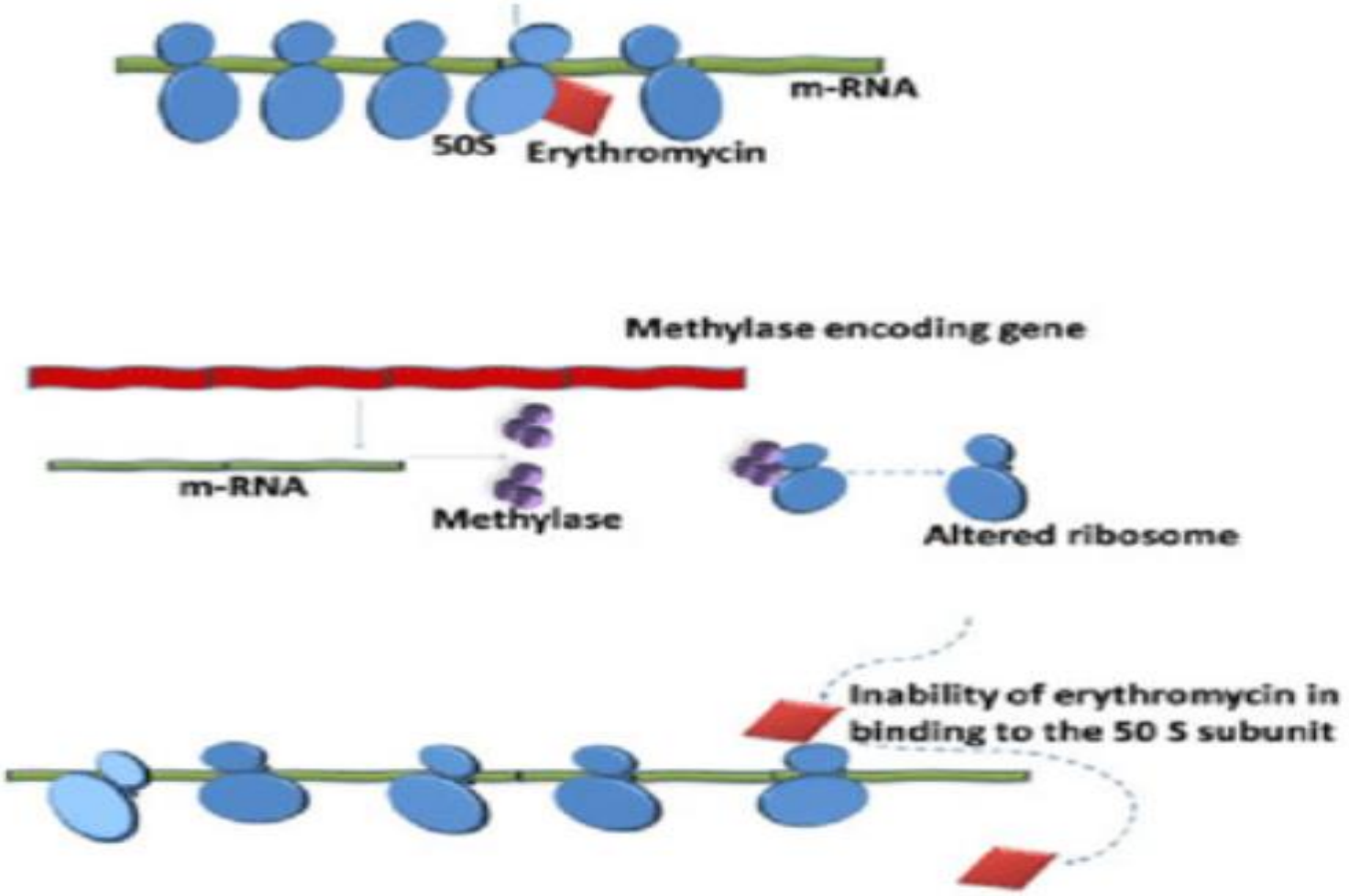
Mechanisms of Tetracycline Inactivation

- **TetX enzyme found in Bacteroides inactivating tetracycline** (it is unusual mechanisms)

3. ALTERED TARGET SITE

- **e.g. Macrolides, Lincosamides, Streptogramins** – disrupts its ability to inhibit protein synthesis and bacterial cell growth, principal mechanism of multiple-agent resistence to MLSb antibiotics among aerobic and anaerobic Grampositive bacteria (e.g. S. aureus, S. pneumoniae, C. perfringens…), may **be located on plasmids or chromosomes**
- ***erm*** (A, B, C) genes determined the **methylases that dimethylate adenine residues on the 23S ribosomal RNA** (50S subunit) **disrupting binding of MLS antibiotic to the ribosome**
- **Oxazolidones (linezolid):** determined by **point mutation within the gene encoding 23S rRNA** of the 50S ribosomal subunit (described in *S. aureus*, *S. epidermidis*, enterococci)
- **Vancomycin resistence: alteration of cell wall precursor in enterococci** determined by **vanA gene (rarely also in staphylococci as VRSA), thickened cell walls in VISA (vankomycin resistant *S. aureus*)**
- **Alteration of target enzymes: mecA** located on **staphylococcal cassete chromosome mec (SCCmec) (located chromosomally) determines alternative PBP2a**
- **PBPs changes also known in betalactams resistant *N. gonorrhoeae, N. meningitidis, S. pneumoniae***
- **Quinolones – mutations in gyrA and/or topoisomerase IV** lead to the resistance in Enterobacteriaceae or P. aeruginosa

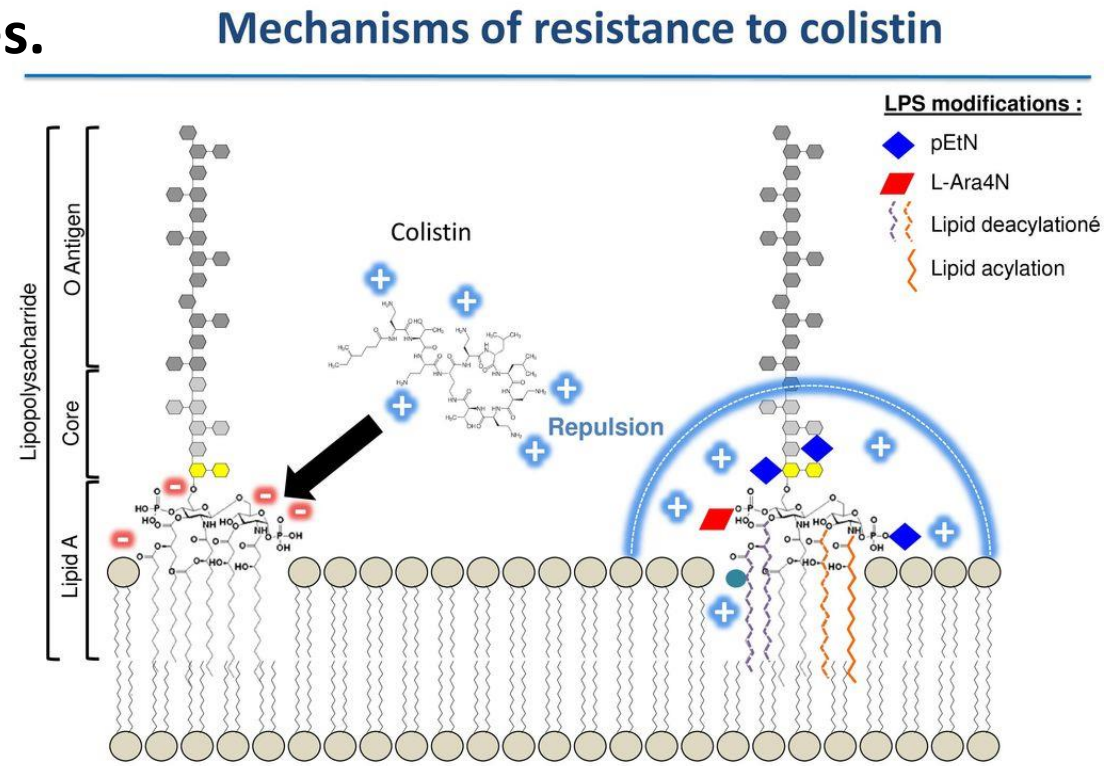
Mechanism of Macrolides, Lincosamides, Streptogramins Resistance



Ref: https://www.researchgate.net/figure/Mechanisms-of-bacterial-resistance-to-macrolide-lincosamide-streptogramin-antibiotics_fig1_257769651

Resitance to colistin in Gramnegative rods

The mechanism of resistance of the MCR gene is a **phosphatidylethanolamine transferase**. The enzyme **transfers** a **phosphoethanolamine** residue **to the lipid A** present **in the cell membrane of gram-negative bacteria**. The altered lipid A has much lower affinity for colistin and related polymyxins resulting in reduced activity of the antimicrobial. Although the same mechanism has been observed before with enzymes like eptA, mcr-1 is the first polymyxin resistance gene known to be capable of **horizontal transfer between different strains of a bacterial species.**

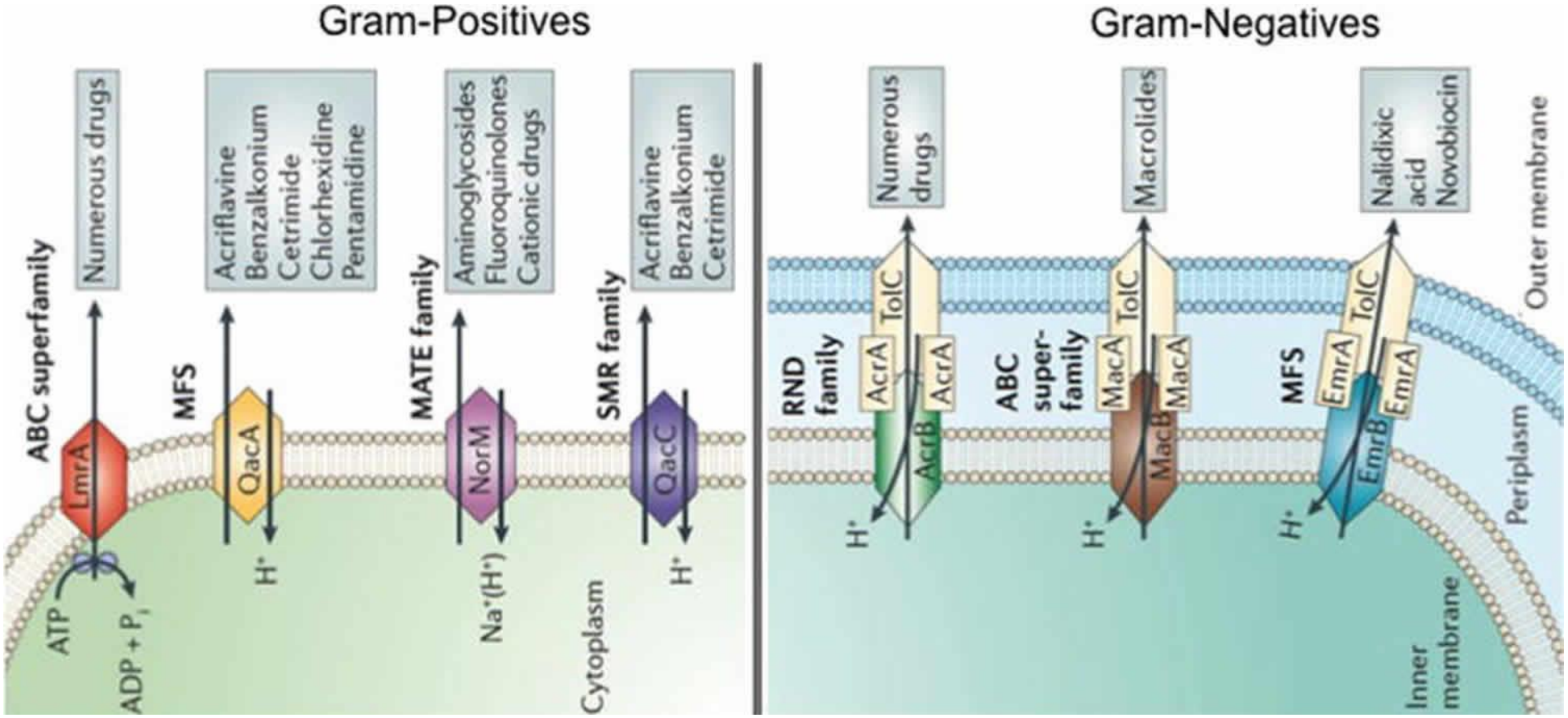


Ref: https://slideplayer.com/slide/12663654/

4. MECHANISMS OF ANTIBIOTIC EFFLUX

- **Specific or multidrug resistance mechanisms decreasing effective concentration of ATB in the bacterial cell**
- **Tetracyclines:** the **most commonly in enteric pathogens** (e.g. *E. coli*, *Shigella*…)
- Energy dependent proces (membrane transporter system)
- The genes (*tet*) located on the **chromosome, plasmids or transposable elements**
- **Macrolides, Streptogramins, Azalides (azithromycin)**: Efflux mechanism mediated by ***mef*** **(in Streptococci)** and ***msrA*** **(in Staphylococci)** are responsible for the resistence
- **Betalactams:** multidrug efflux pump in inner and outer membrane is known in **P. aeruginosa**
- **Fluorochinolones: detected in enteric bacteria and staphylococci, determining by a specific quinolone efflux pump (e.g. EmrAB, AcrAB)**

Representation of different types of efflux pumps in Gram-positive and Gram-negative bacteria



Ref: https://healthjade.net/antibiotic-resistance/

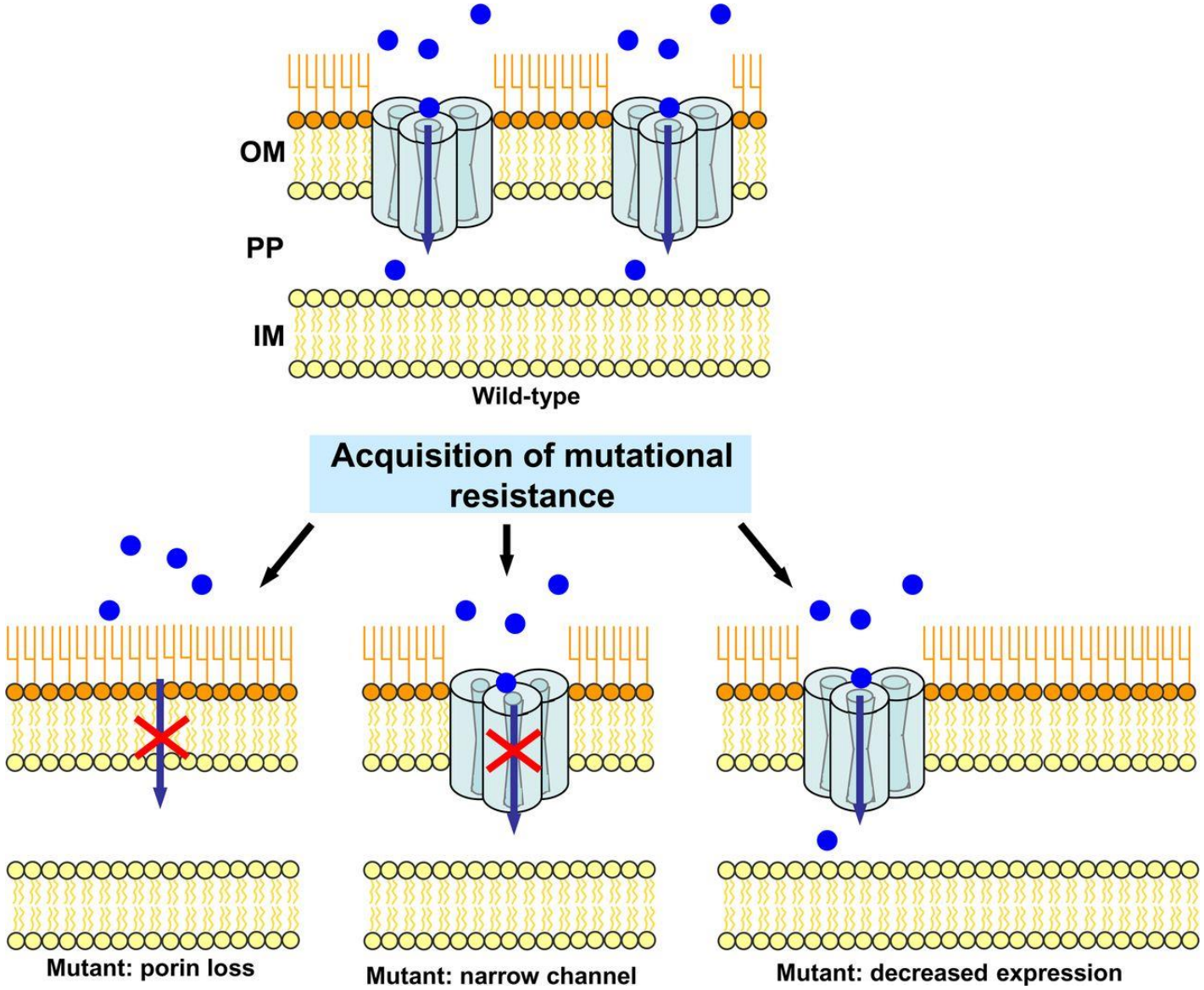
5. DECREASED PERMEABILTY OF BACTERIAL MEMBRANES

- Outer or inner membrane permeability: bacteria can regulate **size** and **number of the porins**

- Relativelly **quickly emerged** (during a particular patient treatment, e.g. imipenem/*P.aeruginosa*)

- **The most known in *P. aeruginosa*** determining rersistance to aminoglycosides, carbapenems

Modification, reduction and loss of porins



Multidrug-resistence mechanisms among bacteria

- Bacterial pathogen can express **more than 1 resistance mechanism** leding to **multidrug-resistance** or even **pan-resistence**
- In general, **resistance in Gramnegative bacteria starts** with limited outer **membrane permeability coupled with the overexpression of MDR efflux pumps**
- **It may alowe to survive bacterial pathogen facilitating the accumulation of new antibiotic-resistrance mutations**
- **Clinically important efflux pumps: RND, MFS, SMR** (staphylococcal multiresistance), **MATE family – such pumps are widespread** among procaryotes responsible for the export of toxic substances and allowing survival in noxious environment
- **Ussually sequentially transfered by multiple-resistant determinants located on mobile genetic elements** (e.g. conjugative transpozon Tn916 conferring resistance to tetracycline na chloramphenicol among bacterial species)
- **Integrons usually capture multiple antibiotic resistance genes,** which can insert **resistence gene cassetes** into their attI integration site and are **often found on transposons carried on plasmids, with seemingly endless recombinant potential**

Ref: Mandell G.L., Bennett J.E., Dolin R. Principles and Practice of Infectious Diseases

Sructure of significant ATB Efflux pumps