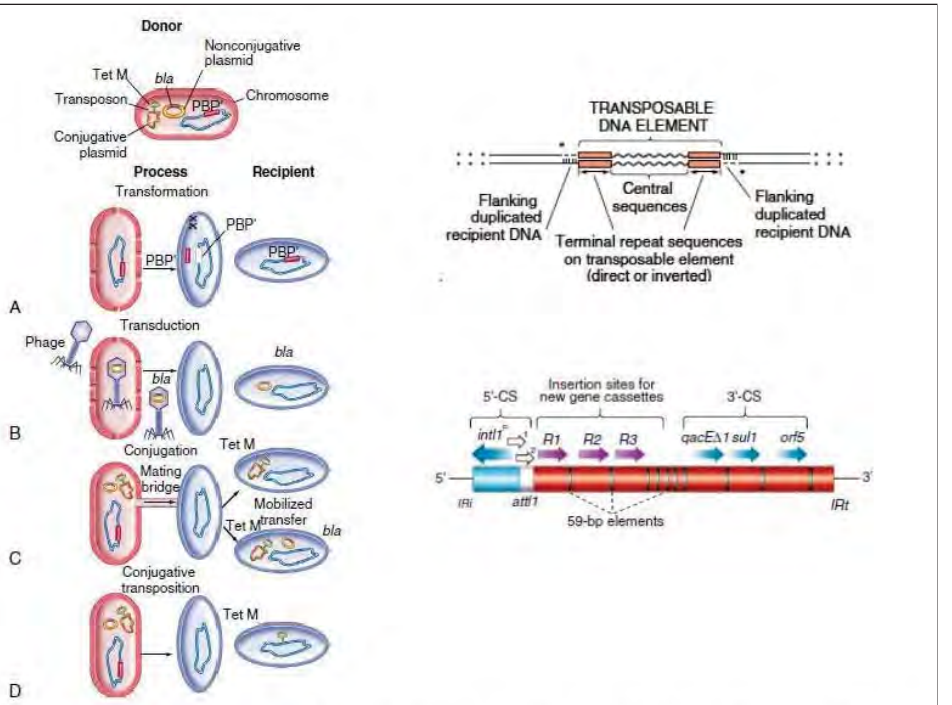
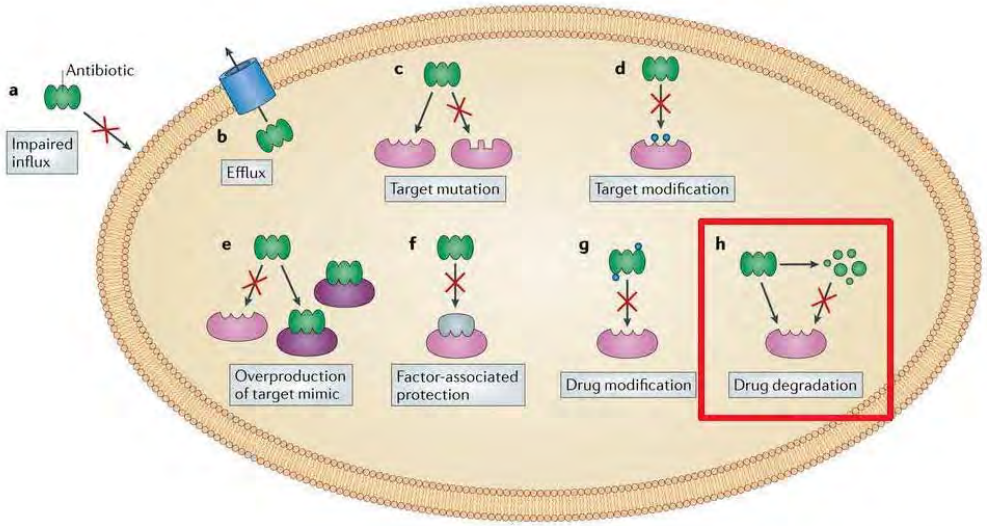
Mechanisms of Antibiotic Resistance

Patcharasarn Linasmita MD
HRH Princess Maha Chakri Sirindhorn Medical Center
Srinakharinwirot University



Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, 18, 235-251

Mechanisms of Antibiotic Resistance



Nature Reviews Microbiology 12, 35–48 (2014)

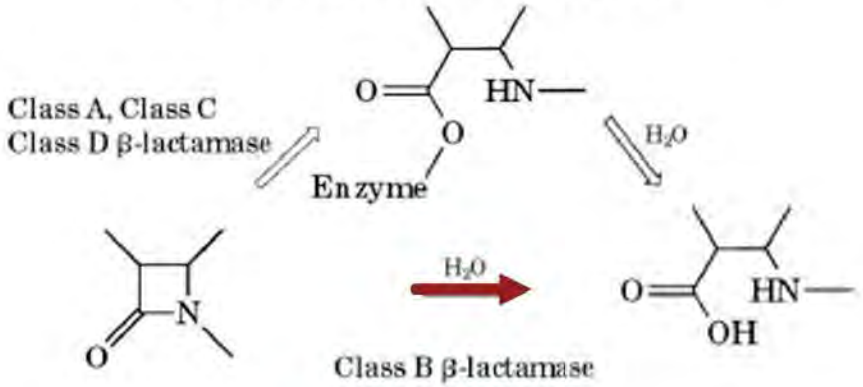
Beta-lactamases

- **Co-evolve** with bacteria as mechanisms of resistance against natural antibiotics
- encoded either by **chromosomal** genes or by transferable genes located on **plasmids** and **transposons**
- ***bla*** genes frequently reside on **integrons**
  - often carry multiple resistance determinants

Ambler Class	Active site	Enzyme type	Substrates
A	Serine	Broad spectrum penicillinases	Benzylpenicillin, aminopenicillins, carboxypenicillins, ureidopenicillins, narrow-spectrum cephalosporins
		Extended spectrum penicillinases	Substrates of broad-spectrum plus oxymino-β-lactams (cefotaxime, ceftazidime, ceftriaxone) and aztreonam
		Carbapenemases	Substrates of extended-spectrum plus cephamycins and carbapenems
B	MBL (Zn^{2+})	Carbapenemases	Substrates of extended-spectrum (**not include aztreonam**) plus cephamycins and carbapenems
C	Serine	Cephalosporinases	Substrates of extended-spectrum plus cephamycins
D	Serine	Broad spectrum oxacillinases	Aminopenicillins, ureidopenicillin, cloxacillin, methicillin, oxacillin, and some narrow-spectrum cephalosporins
		Extended spectrum oxacillinases	Substrates of broad-spectrum plus oxymino-β-lactams and monobactams
		Carbapenemases	Substrates of extended-spectrum plus cephamycins and carbapenems

Group	Distictive Substrates	Inhibited by CLAV	Inhibited by EDTA	Molecular class
1	Cephalosporins	No	No	C
1e	Cephalosporins	No	No	C
2a	Penicillins	Yes	No	A
2b	Pen, early ceph	Yes	No	A
2be	Ext Sp Ceph, mono	Yes	No	A
2br	Penicillins	No	No	A
2ber	Ext Sp Ceph, mono	No	No	A
2c	Carbenicillin	Yes	No	A
2ce	Carbeni, cefepime	Yes	No	A
2d	Cloxacillin	Variable	No	D
2de	Ext Sp Ceph	Variable	No	D
2df	Carbapenems	Variable	No	D
2e	Ext Sp Ceph	Yes	No	A
2f	Carbapenems	Variable	No	A
3a	Carbapenems	No	Yes	B
3b	Carbapenems	No	Yes	B

Serine-based β-lactamase:
hydrolyze the β-lactam ring through
a serine residue at active site



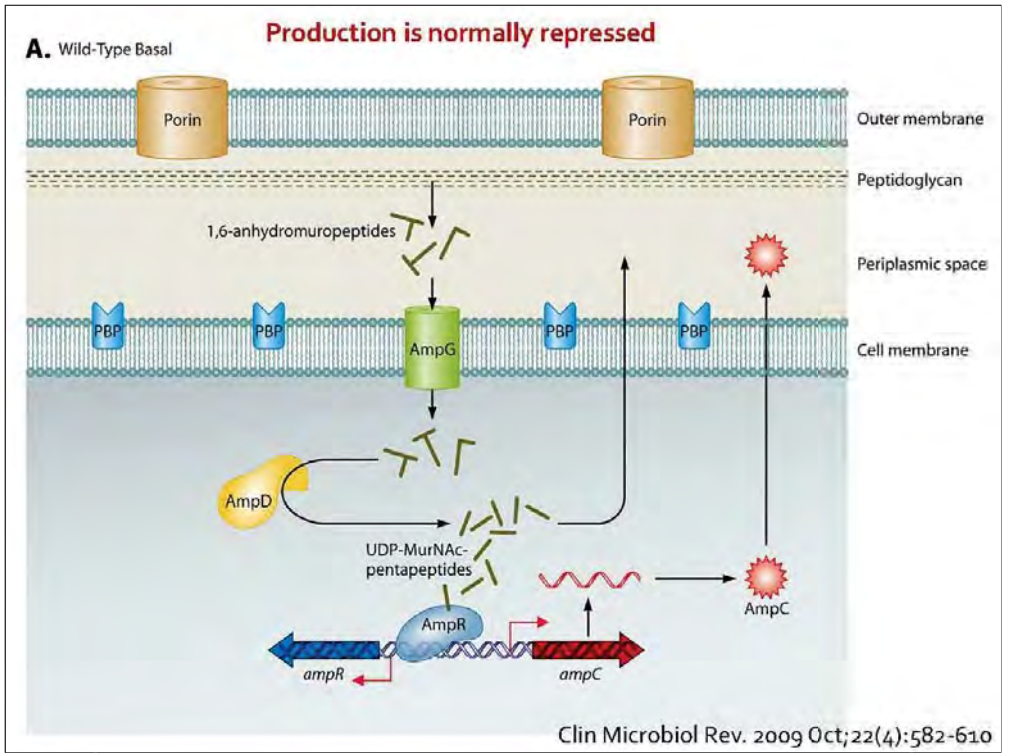
Metallo β-lactamase:
Use Zn^{2+} to break the amide bond

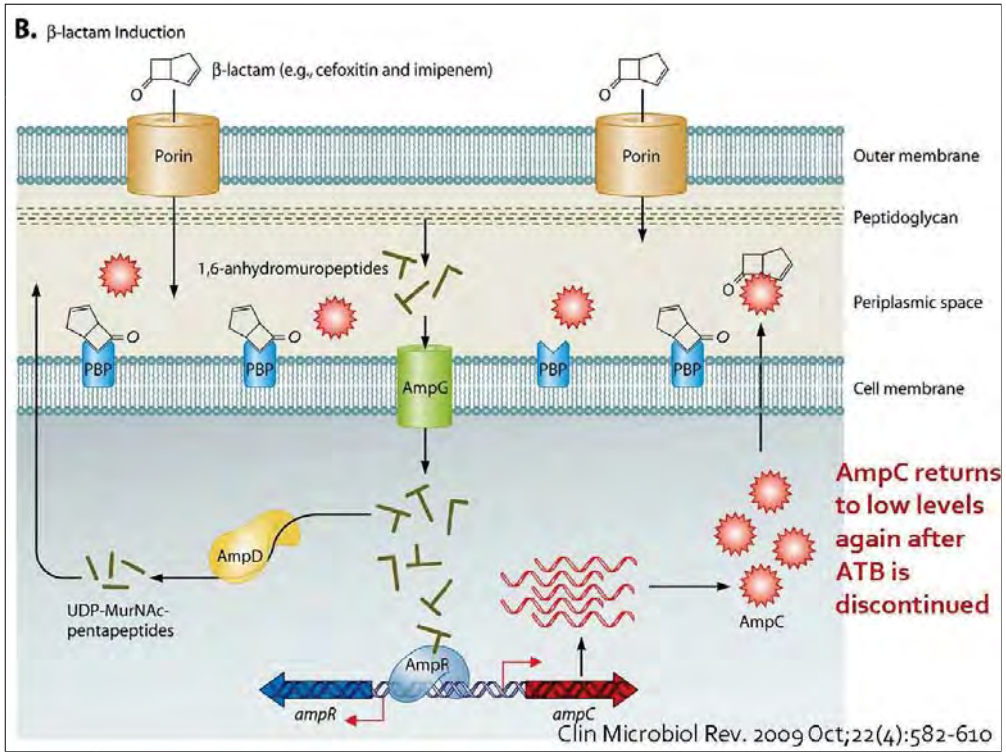
AmpC Enzymes

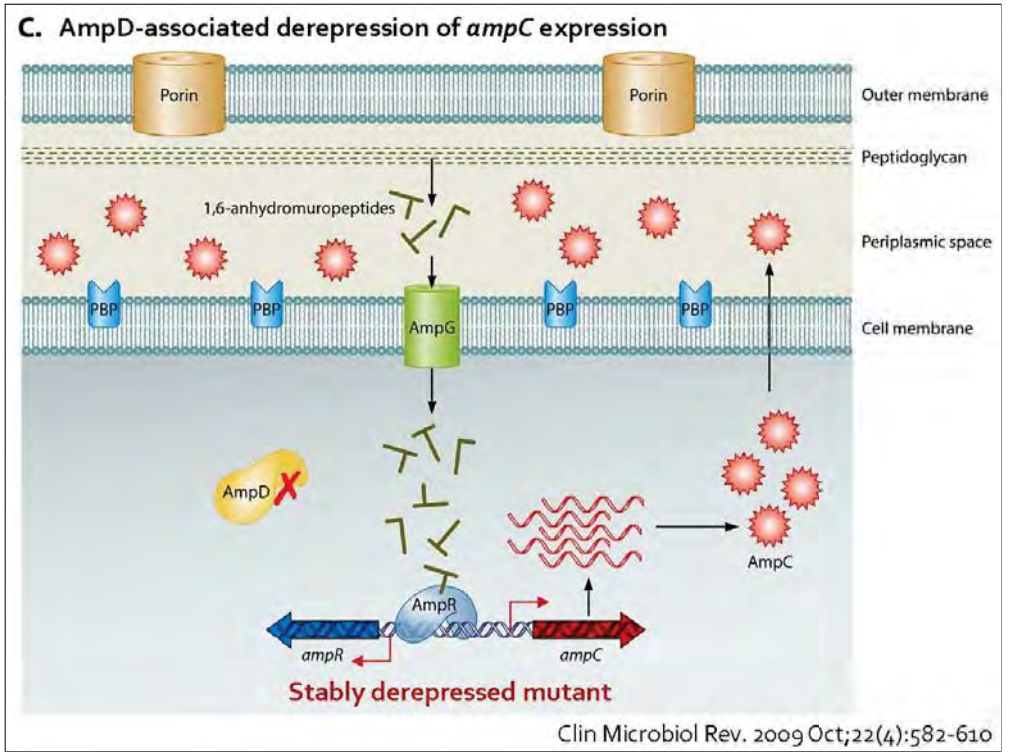
- Molecular class C, functional group 1
- Primarily **chromosomal** enzymes
  - Also in plasmids
- Confer resistance to penicillins, narrow-spectrum cephalosporins, oxymino-β-lactams, and cephamycins
  - Cefepime and aztreonam are usually poor substrates
- Not susceptible to β-lactamase inhibitors

AmpC Enzymes

- Production in GNB is normally repressed
- A transient increase in production (10x – 100X) can occur in **the presence of β-lactam ATBs** in species that possess **inducible AmpC enzyme**
  - *Enterobacter*, *Citrobacter freundii*, *Serratia*, *M. morganii*, *Providencia*, indole-positive *Proteus*, and *P. aeruginosa*
    - AmpC beta-lactamase production returns to low levels again after antibiotic exposure is discontinued
  - mutations in the *ampD* locus of the gene, leading to permanent hyperproduction (derepression) of enzymes
    - Stably derepressed mutant







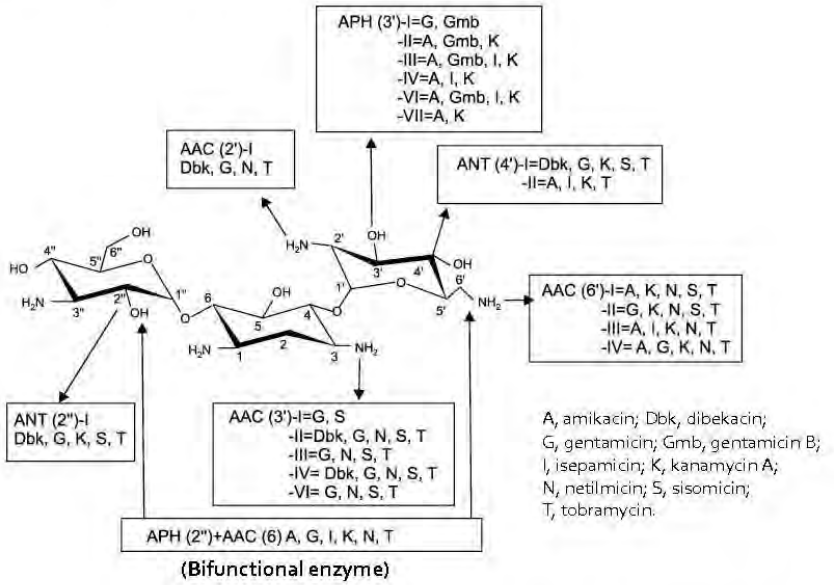
Mechanisms of Aminoglycosides Resistance

- **Enzymatic inactivation**
  - **Most common**
- Alteration of ribosome
  - common
- Efflux pump
- Decrease permeability
  - Alteration of membrane electrical charge
  - Lack of a proton motive force

Modification of Aminoglycosides

- Three main classes of enzyme
  - **Acetyltransferase** : acetylation (AAC)
    - *Enterobacteriaceae,*
    - *Pseudomonas aeruginosa, Acinetobacter baumannii*
    - *Providencia, Proteus*
    - *Staphylococcus*
  - **Phosphotransferase** : phosphorylation (APH)
    - *Staphylococcus,* ***Streptococcus***
    - *Enterobacteriaceae, Pseudomonas aeruginosa*
  - **Nucleotidyltransferase** : adenylylation (ANT)
    - *Enterobacteriaceae, Pseudomonas aeruginosa*
    - *Staphylococcus*

Aminoglycoside-modifying enzymes and their substrates

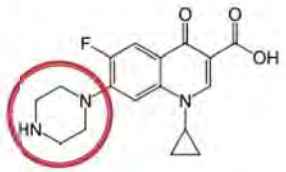


(Bifunctional enzyme)

Korean J Clin Microbiol. 2009 Jun;12(2):57-61

Bifunctional Enzymes

- AAC(6′)APH(2″)
  - *Staphylococcus,* ***Enterococcus***
  - Acetylation of aminoglycosides
  - Phosphorelation of aminoglycosides
- AAC(6′)-Ib-cr
  - *Enterobacteriaceae*
  - Acetylation of aminoglycosides
  - Acetylation of **quinolones**
    - **at piperazine ring**



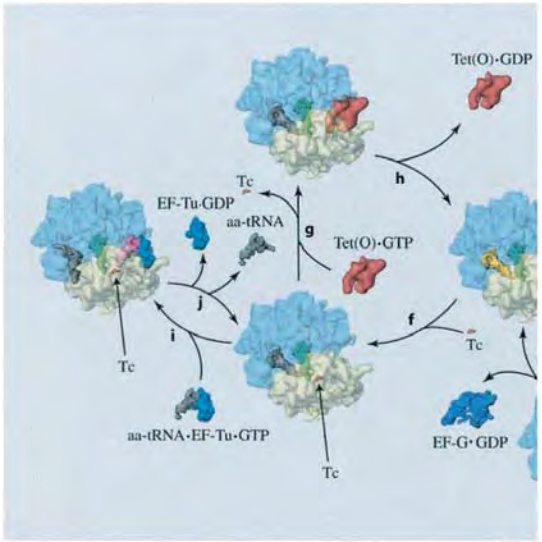
Aminoglycosides resistance: Ribosomal Level (Altered Target Sites)

- Methylation of 16S rRNA (component of 30S)
  - In *Enterobacteriaceae* and nonfermenting GNB
  - **Plasmid** mediated
    - Gene: ***armA***, *rmtA*, *rmtB*, *rmtC*, *rmtD*, *rmtE*, *npmA*
  - Major mechanism of resistance to aminoglycosides
- Mutation of the S12 protein of the 30S subunit

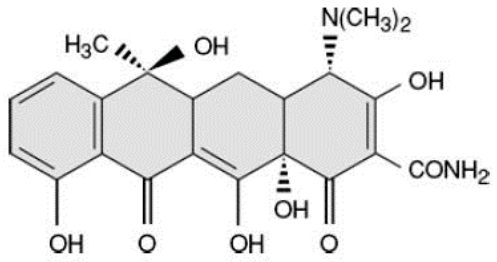
Tetracycline resistance mechanisms

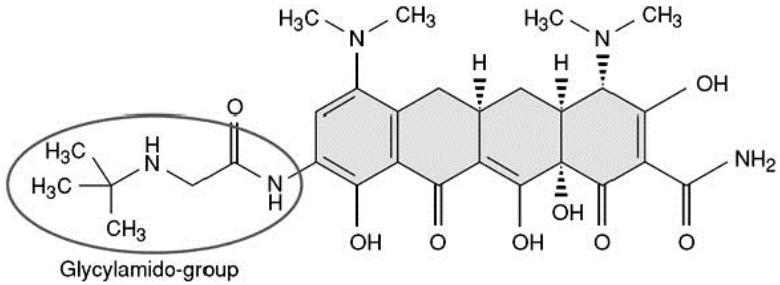
- **Efflux pump** (most common)
  - TetA-L
- **Ribosomal protection** (common)
  - **TetM, TetO**
- Less common mechanisms
  - Enzymatic inactivation
    - TetX, Tet34, Tet37 in *Bacteroides, Vibrio*
  - Mutation in 16S rRNA
    - Found in *Helicobacter pylori*

Ribosomal protection



Antimicrob Agents Chemother. 2003 Dec;47(12):3675-81





Macrolide resistance mechanisms *Streptococcus / Staphylococcus*

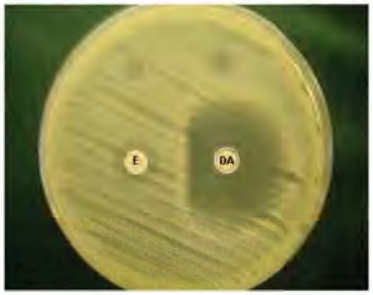
- Alteration of target ribosomal binding site
  - **Methylation -> MLS$_B$ phenotype**
    - *erm* gene
  - L4/L22 mutation (*Streptococcus pneumoniae*)
- Efflux
  - **M phenotype -> *mef*** gene (*Streptococcus*)
    - *mef(A)* -> GAS, *mef(E)* -> (*Streptococcus pneumoniae*)
  - MS$_B$ phenotype -> *msr* gene (*Staphylococcus*) - rare
- Enzyme inactivation - rare
  - M phenotype -> ere gene (*Staphylococcus*)

MLS$_B$ Phenotype (*Streptococcus/Staphylococcus*)

- Pattern: **M**acrolide, **L**incosamide, **S**treptogramin B (quinupristin)
- *erm* (erythromycin ribosome methylation) gene
  - **dimethylation of adenine residues on the 23S rRNA of the 50S subunit**
  - *erm(A) – Strep/Staph; erm(B) – Strep; erm(C) – Staph*
- Constitutive - cMLS$_B$
- Inducible - iMLS$_B$
  - *Streptococcus pyogenes, Staphylococcus aureus*
  - *Streptococcus pneumoniae* – less likely

Inducible MLS$_B$ Phenotype (*erm* gene)

- *Staphylococcus*
  - Induced MLS$_B$ phenotype **only** by **macrolide**
- *Streptococcus*
  - Induced by lincosamide and macrolides



Resistance Mechanisms in *Streptococcus pneumoniae*

- Alteration of target site
  - **Mosaic PBP / mutation in PBP -> PRSP or CRSP**
  - dihydrofolate reductase -> Trimethoprim
  - dihydropteroate synthase -> Sulfonamide
  - Methylation of ribosome -> MLS$_B$
  - DNA gyrase / topoisomerase IV -> quinolones
- Protection of ribosome from tetracycline
- Efflux

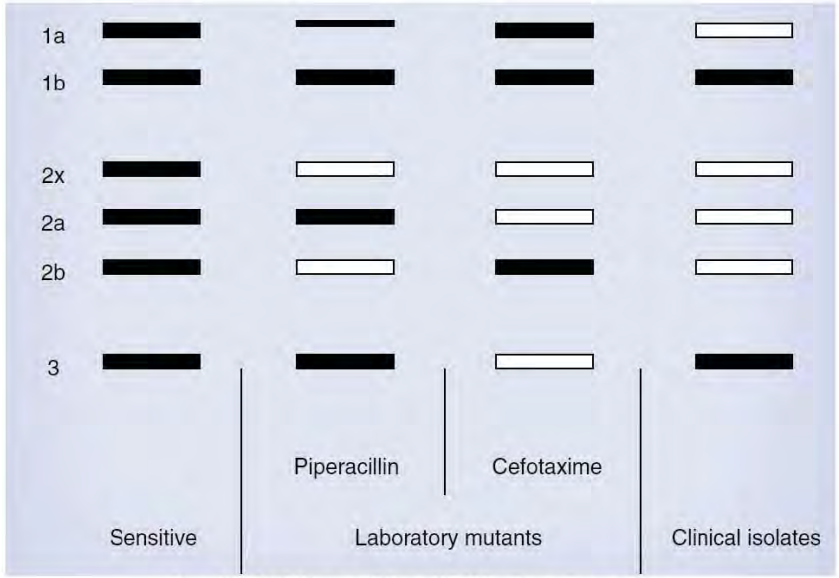
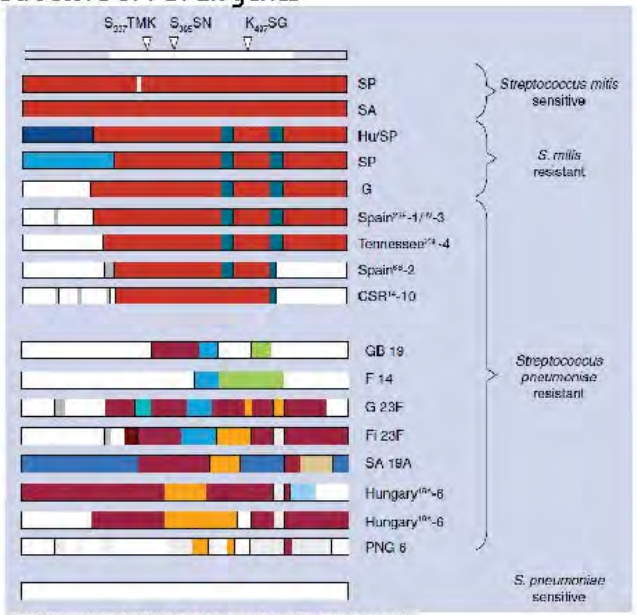


Figure 1. Penicillin-binding protein profiles of *Streptococcus pneumoniae*.
A summary of all changes that have been described in different mutants or clinical isolates are shown. The six are marked on the left. White: low-affinity variants in resistant laboratory mutants (selected with piperacillin or cefotaxime) and clinical isolates; thin line: less PBP1a. In particular, for cefotaxime-resistant mutants, a decrease in the amount of a low-affinity PBP2x has been described [71].
PBP: Penicillin-binding protein.

Future Microbiol. 2012 Mar;7(3):395-410

Mosaic structure of PBP2x genes



Future Microbiol. 2012 Mar;7(3):395-410

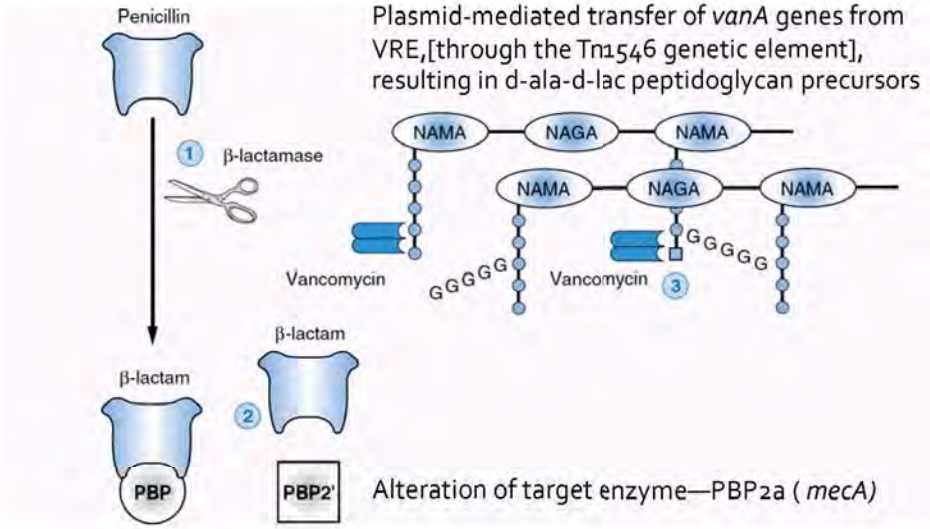
Mechanisms of Beta-lactam resistance in *Streptococcus pneumoniae*

- Mosaic PBP genes
  - Common gene pool accessible to *S. pneumoniae*, *S. mitis*, *S. oralis*
  - Recombination and horizontal gene transfer
- Mutations in PBP genes
  - PBP2x, PBP2b – low level resistance
  - PBP1a – high level resistance
    - Required the presence of altered PBP2x, PBP2b

Mosaicism in PBP also found in

- *Neisseria gonorrhoeae*
  - *penA* gene
    - Gene encoded PBP
- *Haemophilus influenzae*
  - *ftsI* gene
    - Gene encoded PBP3

Resistance Mechanisms in *Staphylococcus aureus*



MRSA, *mecA* gene, and SCCmec

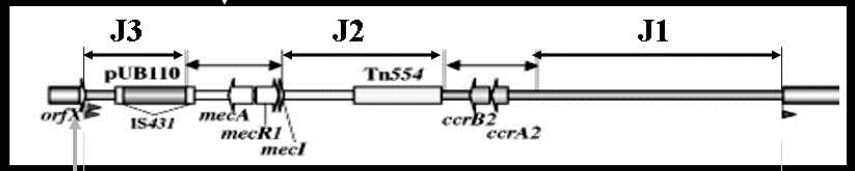
- *mecA* encodes **PBP2a**
  - **PBP2a** has a **reduced affinity** for beta-lactam
    - However, **ceftaroline** has a high affinity for PBP2a
- *mecA* is in the SCCmec genetic element of MRSA
- SCCmec – mobile genetic element
  - *mec* gene complex
  - *ccr* gene complex – responsible for the mobility
  - have characteristic directly-repeated nucleotide sequences (DRs) and inverted-complementary sequences (IRs) at both ends

The essential structure of SCC*mec* elements

1. *mec* gene complex **2. *ccr* gene complex**

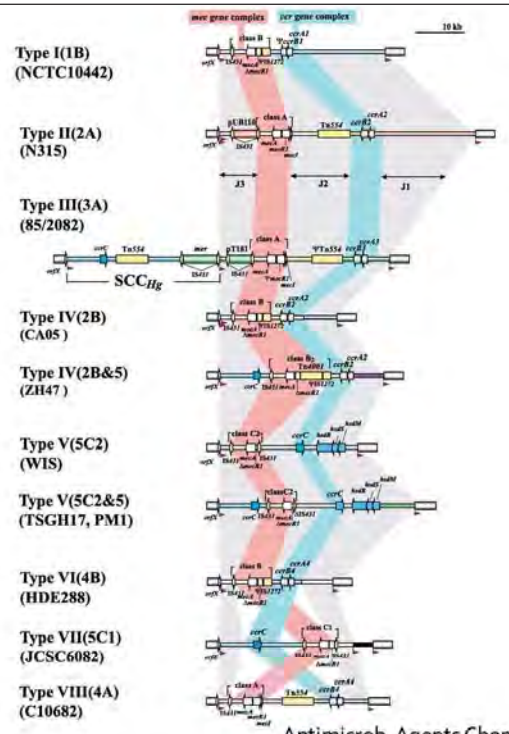
Class A
B
C
D

type-1 (*ccrA1ccrB1*)
2 (*ccrA2ccrB2*)
3 (*ccrA3ccrB3*)
4 (*ccrA4ccrB4*)
5 (*ccrC*)

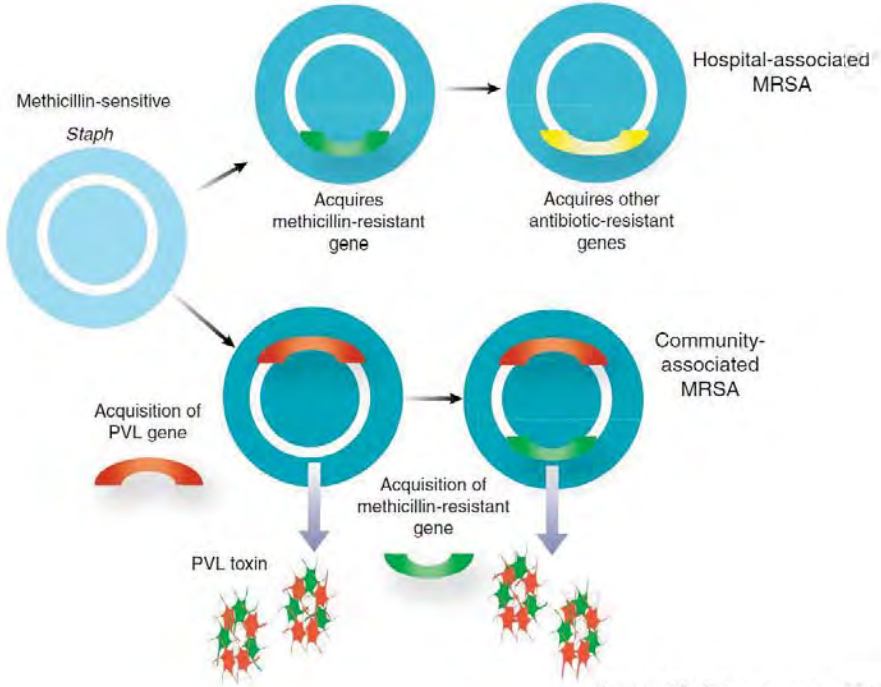


3. Direct repeats/ Inverted repeats
4. integrated at the 3' end of *orfX*

http://www.staphylococcus.net/



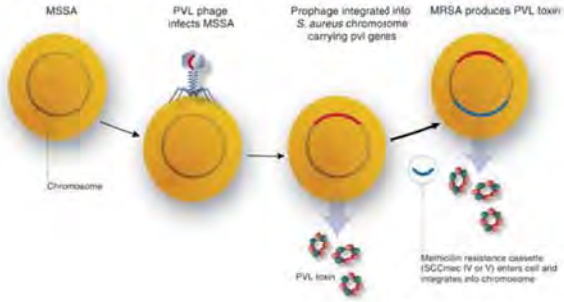
Antimicrob. Agents Chemother. December 2009 vol. 53 no. 12 4961-4967



Nature Medicine 13, 522 - 523 (2007)

MRSA

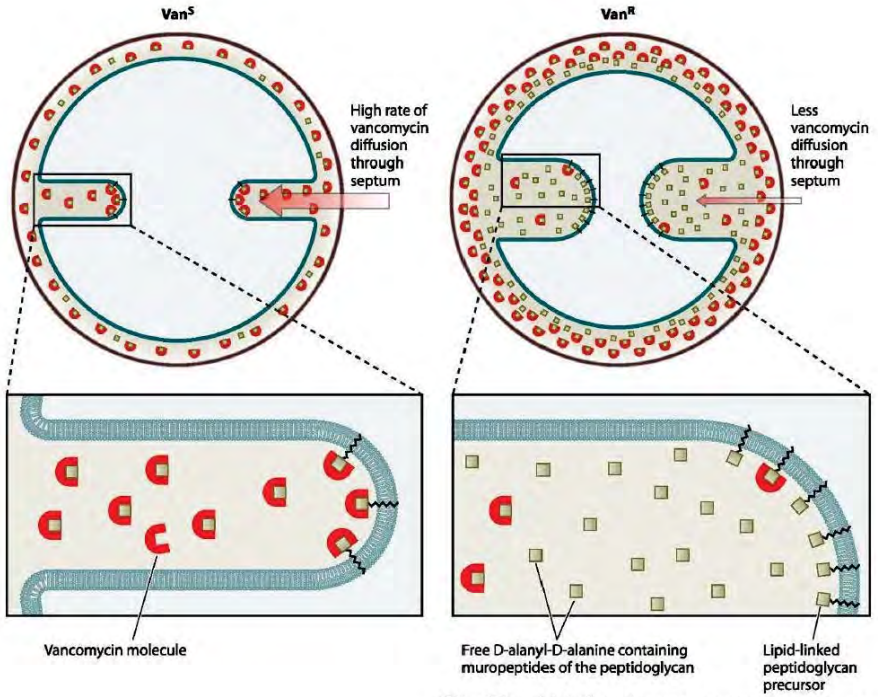
- HA-MRSA
- CA-MRSA
  - SCCmec type IV, V
  - Frequently associated with PVL



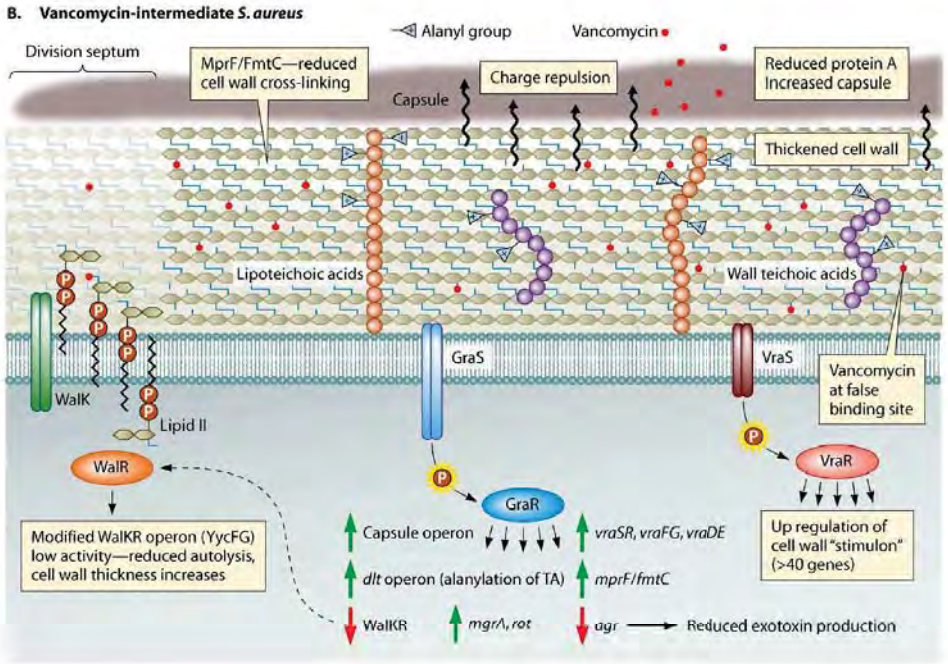
Laboratory Investigation (2007) 87, 3–9.

Vancomycin-Intermediate *S. aureus* (VISA)

- Unusually **thick peptidoglycan cell wall**
  - **Less completely cross-linked**
  - Increased number of **false binding site** for vanco
    - Nonamidated glutamine precursor
      - Vancomycin molecules are absorbed to these sites



Clin. Microbiol. Rev. January 2010 vol. 23 no. 1 99-139



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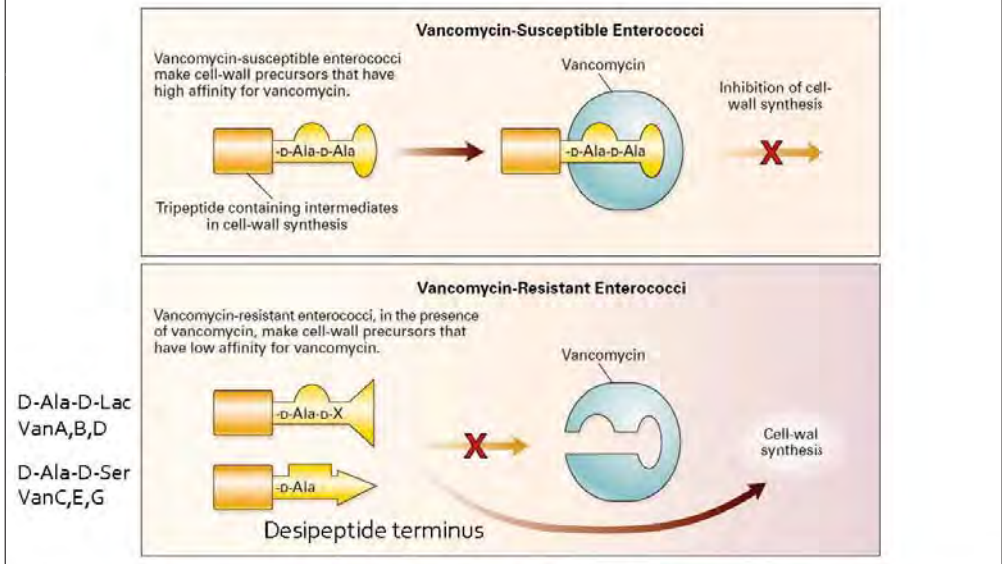


Vancomycin-susceptible enterococci synthesize cell-wall precursors ending in D-Ala-D-Ala, which, after translocation from the cytoplasm to the cell surface, bind vancomycin with high affinity; once bound, these precursors cannot participate in cell-wall synthesis. Vancomycin-resistant enterococci, in the presence of an inducer like vancomycin, generate precursors with different termini (D-Ala-D-Lac, D-Ala, or D-Ala-D-Ser), which have low affinity for vancomycin[33-36] and thus can continue, in large part, to be used to synthesize cell wall. Ala denotes alanyl or alanine, and X lactate for VanA, VanB, and VanD types of resistance and serine for VanC and VanE types.
VanG

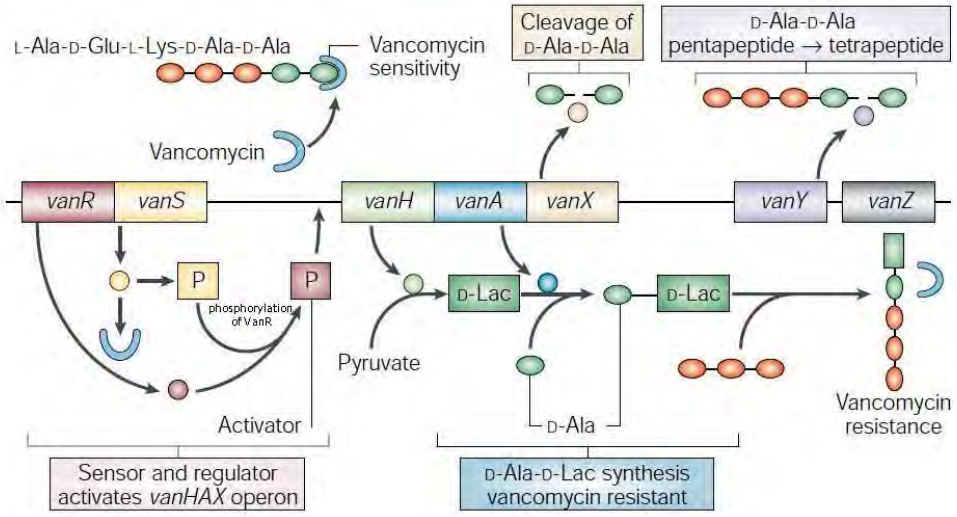
N Engl J Med 2000; 342:710-721

	A	B	C	D	E	G
Vanco MIC (ug/ml)	64 to > 500	4 to > 500	2 – 32	64 – 128	16	12 – 16
Teico MIC (ug/ml)	16 to > 500	0.5 – 2	0.5 – 2	4 – 64	0.5	0.5
Expression	**Inducible**	**Inducible**	Constitutive, **inducible**	Constitutive	**Inducible**	Not described
Gene Location	**Plasmid**, Chromosome	**Plasmid**, Chromosome	Chromosome	Chromosome	Chromosome	Chromosome
Ligase gene	***vanA***	***vanB***	*vanC*	*vanD*	*vanE*	*vanG*
Terminus	d-ala-d-**lac**	d-ala-d-**lac**	d-ala-d-**ser**	d-ala-d-**lac**	d-ala-d-**ser**	d-ala-d-**ser**
Common species	*E. faecalis*, *E. faecium*, *S. aureus*	*E. faecalis*, *E. faecium*	*E. gallinarum* (*vanC-1*), *E.casseliflavus* (*vanC-2*), *E. flavescens* (*vanC-3*)	*E. faecium*	*E. faecalis*	*E. faecalis*

Most **vanB**-containing isolates are **susceptible to teicoplanin** on testing, but the development of resistance in vivo and in vitro has been documented

N Engl J Med 2000; 342:710-721
Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, 18, 235-251

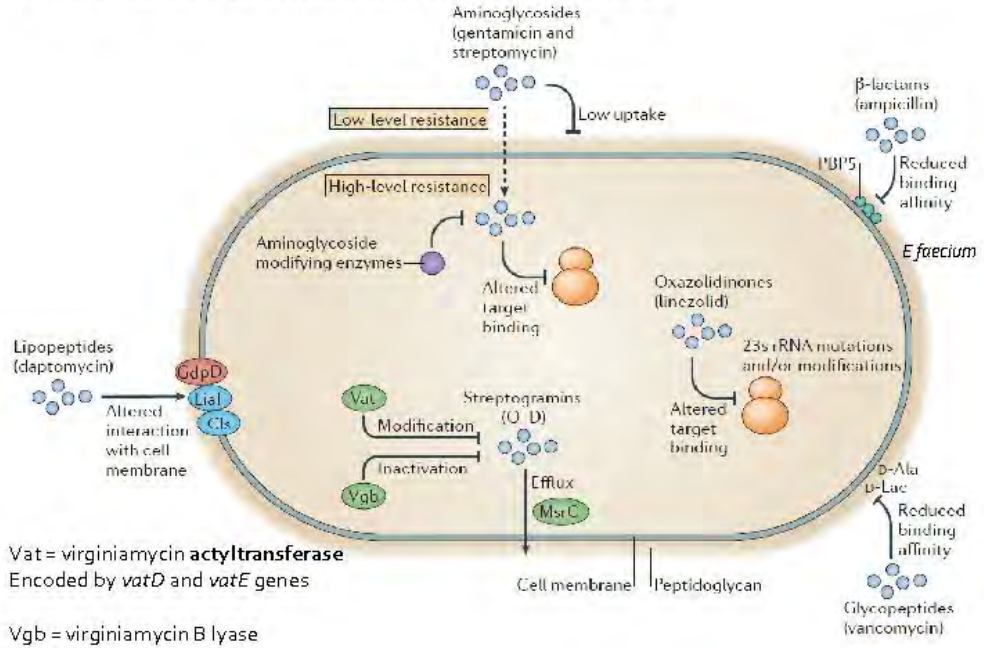
A TWO-COMPONENT REGULATORY system VanR–VanS regulates vancomycin resistance in VRE and VRSA strains



VanH = a dehydrogenase
VanA = a ligase
VanX = a dipeptidase
VanY = a carboxypeptidase

Nature Reviews Genetics 4, 432-441 (June 2003)

Main Mechanisms of Enterococcal Antibiotic Resistance



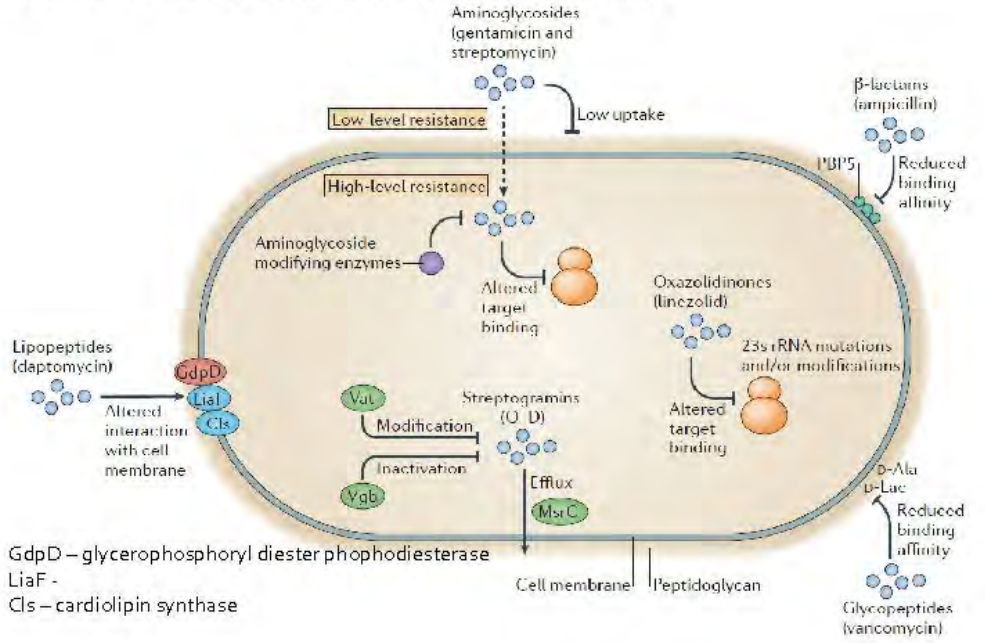
Vat = virginiamycin **acetyltransferase**
Encoded by *vatD* and *vatE* genes

Vgb = virginiamycin B lyase

MsrC = macrolide–streptogramin resistance protein

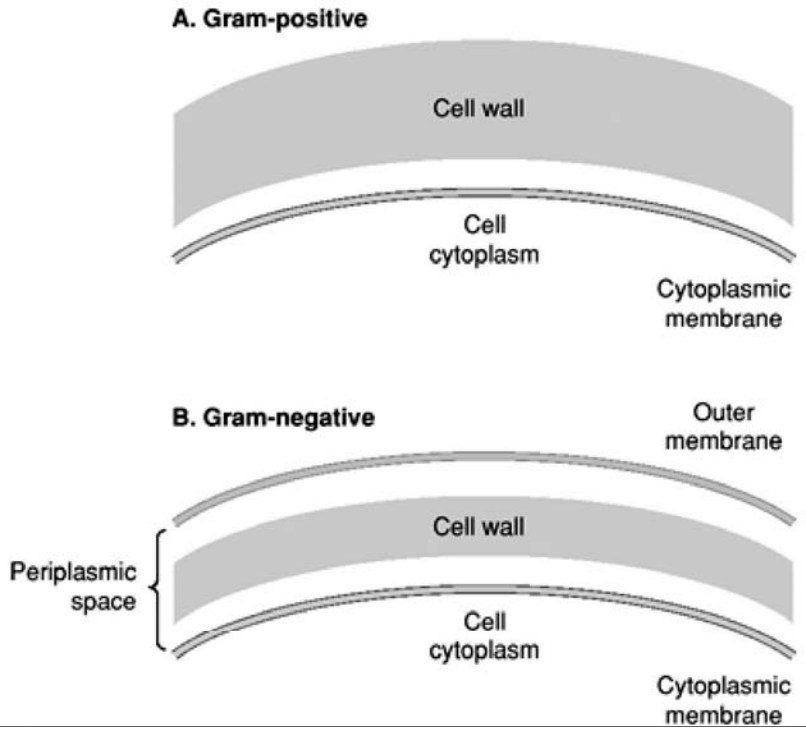
Nature Reviews Microbiology 10, 266-278

Main Mechanisms of Enterococcal Antibiotic Resistance



GdpD – glycerophosphoryl diester phophodiesterase
LiaF -
Cls – cardiolipin synthase

Nature Reviews Microbiology 10, 266-278

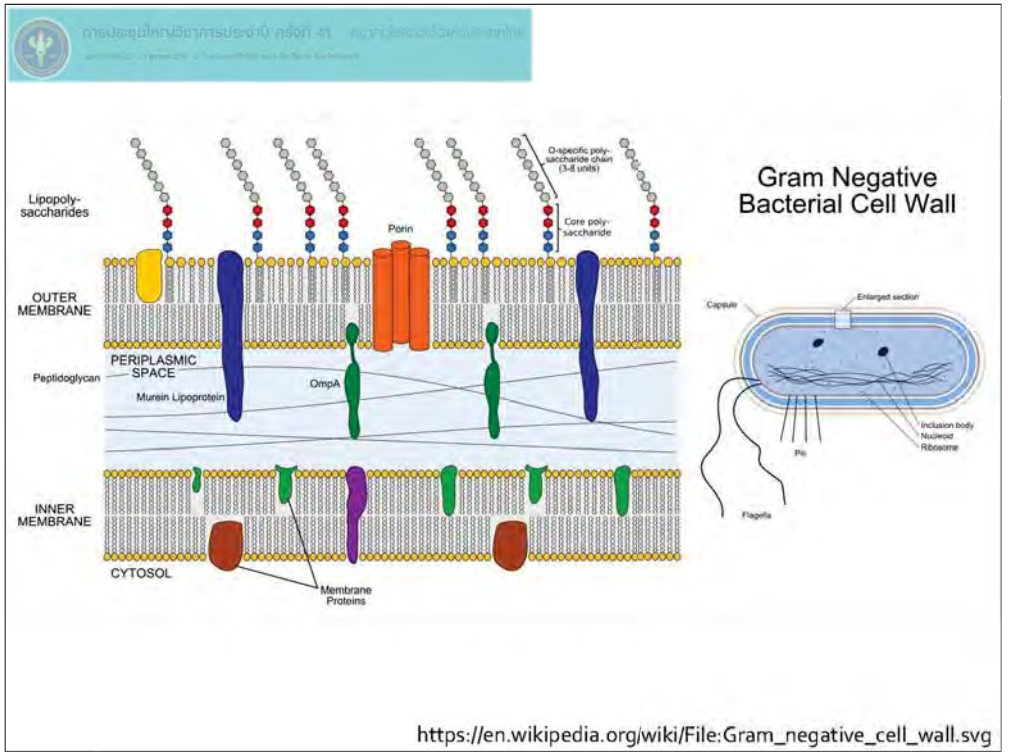


Decrease Membrane Permeability

- Outer Membrane permeability (Gram-neg)
  - Porin – passage for hydrophilic molecules
    - Non specific
      - OmpF - larger porins; OmpC - smaller porins
    - Substrate specific
      - **OprD** – channel for carbapenems *in P. aeruginosa*
  - **Larger molecules, more neg charged, hydrophobicity ->> "less permeability"**
- Inner Membrane permeability
  - Net electrical charge and a proton motive force

Uptake of Aminoglycoside

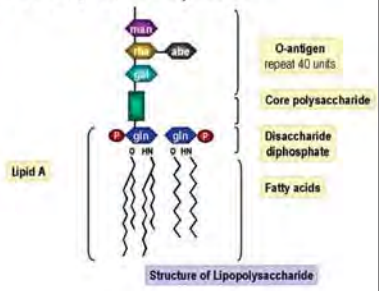
- AG retain **positive** charge
- Being "pulled" across the inner membrane
  - Depend on level of **internal negative charge**
  - "proton motive force"
- Energy dependent
  - **Oxidative** metabolism is essential
    - **Facultative** organisms grown **anaerobically** are resistant to aminoglycoside
      - **a lack of a proton motor force**
      - marked reduction of drug uptake



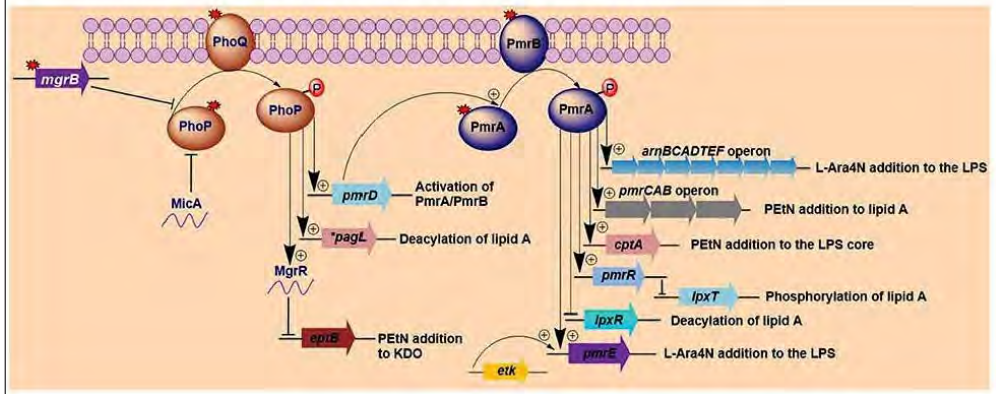
https://en.wikipedia.org/wiki/File:Gram_negative_cell_wall.svg

Acquired Colistin / polymyxin resistance in *Enterobacteriaceae*

- Lipopolysaccharide modifications
  - PmrA/PmrB and PhoP/PhoQ two-component system-mediated **LPS modifications**
    - The addition of **phosphoethanolamine (PEtN)** and **4-amino-4-deoxy-L-arabinose (L-Ara4N)** to lipid A
      - Reduction in net negative charge
- Trapped by capsular polysaccharide
- Efflux pump

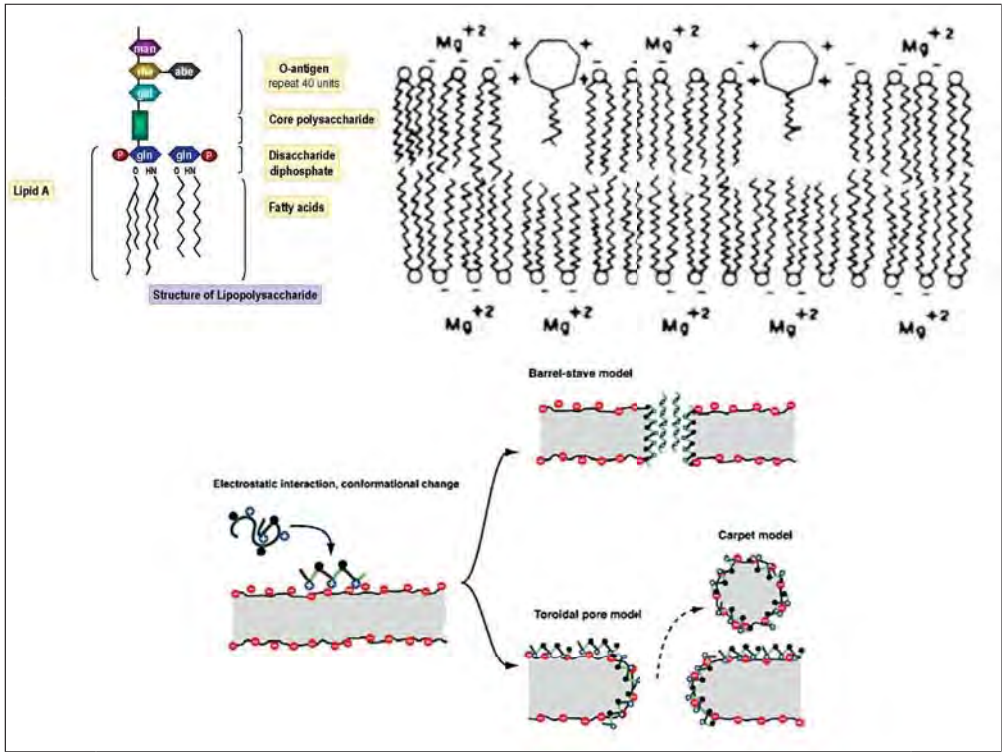


Activation of lipopolysaccharide-modifying genes involved in Polymyxin resistance in Gram-negative bacteria



The addition of **phosphoethanolamine (PEtN)** and **4-amino-4-deoxy-L-arabinose (L-Ara4N)** to lipid A
->>> Reduction in net negative charge

Front Microbiol. 2014 Nov 26;5:643

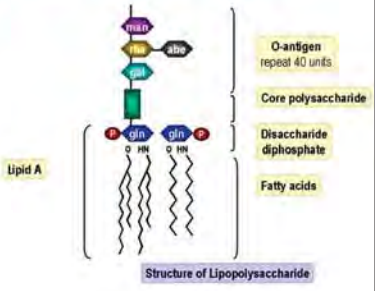


Acquired Colistin / polymyxin resistance in *P aeruginosa*

- PmrA/PmrB and PhoP/PhoQ two-component system-mediated LPS modifications
- **Other** two-component systems
  - The ColR/ColS and CprR/CprS TC regulatory systems
- Overexpression of the outer membrance protein **"OprH"**
  - A protein that binds to **divalent cation-binding sites of LPSs**
    - making these sites unavailable for polymyxin binding
      - Overexpression alone is not sufficient for resistance

Acquired Colistin / polymyxin resistance in *Acinetobacter*

- PmrA/PmrB two-component system-mediated LPS modifications
- **"Loss of LPS"**-mediated colistin resistance
  - **Mutation** in in the first three **lipid A biosynthesis** genes (***lpxA***, ***lpxC***, and ***lpxD***)
    - inactivation with the insertion of **ISAba11 element**
      - "insertional inactivation"



OprH replaces outer membrane stabilizing divalent cations

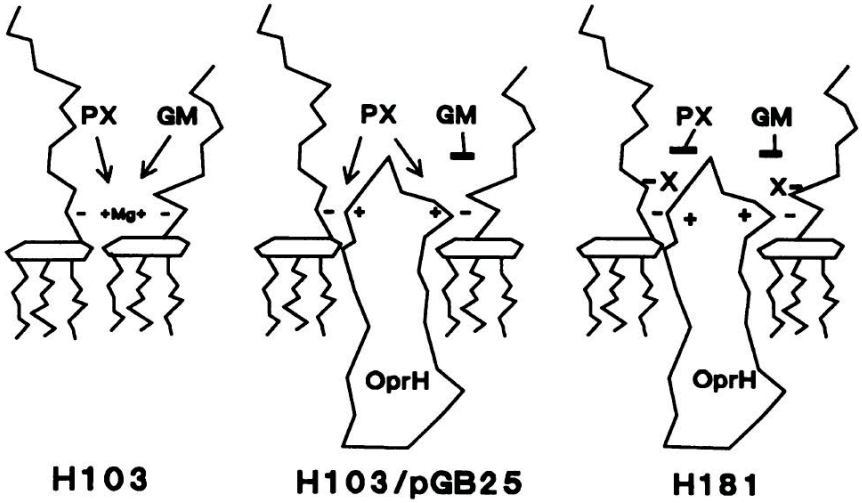
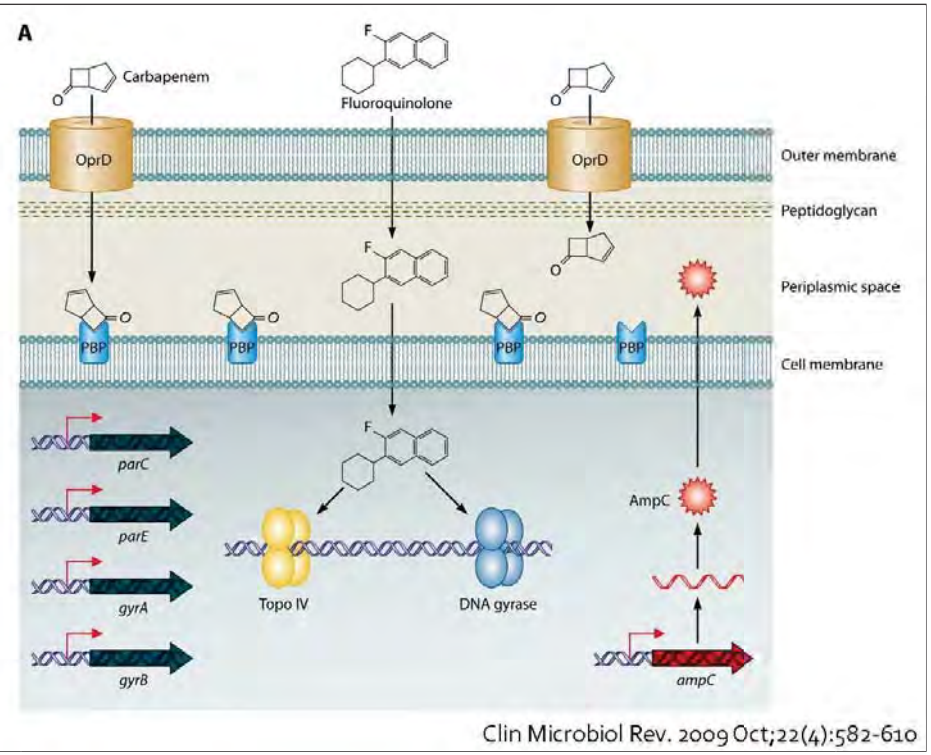
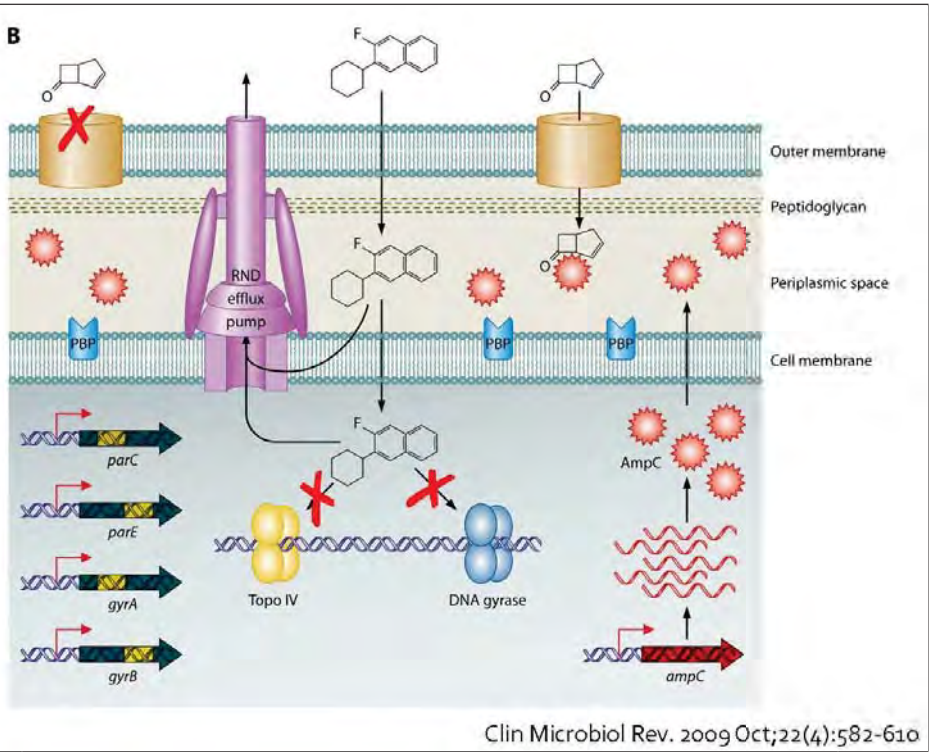


FIG. 6. Model explaining the influence of OprH and LPS on susceptibility to gentamicin (GM), EDTA (not shown but predicted to be the same as GM), and polymyxin B (PX). The model proposes three types of surface sites. Strain H103 would contain the normal divalent cation binding sites on LPS, with divalent cations, such as Mg^{2+}, cross-bridging and stabilizing adjacent LPS molecules. Such sites have been shown to be the sites of interactions of PX, GM, and EDTA (15). In strain H103/pGB25, we propose that OprH replaces divalent cations in the outer membrane, leading to resistance to GM and EDTA. However, such sites are proposed to still permit the interaction of PX (perhaps because of its hydrophobic tail) with its LPS binding site. A similar situation would prevail in the variants of strain H181 with LPS alterations. In strain H181, we propose that there is an LPS alteration (indicated as X) in addition to OprH overexpression, resulting in PX resistance.

J Bacteriol. 1991 Nov;173(21):6657-64

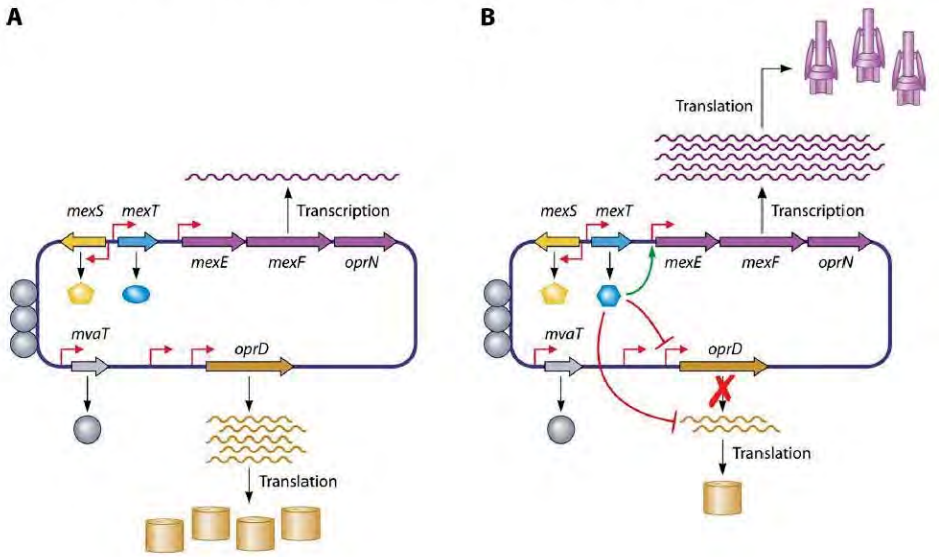


Clin Microbiol Rev. 2009 Oct;22(4):582-610

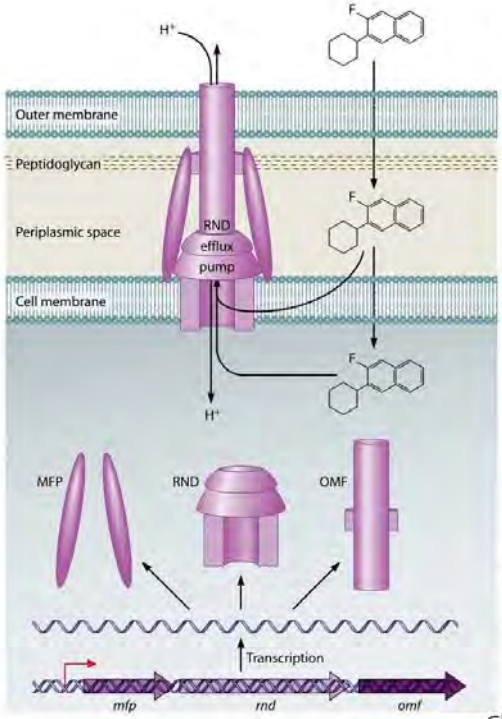


Clin Microbiol Rev. 2009 Oct;22(4):582-610

Coregulation of *mexEF-oprN* and *oprD* in *P. aeruginosa*



Clin Microbiol Rev. 2009 Oct;22(4):582-610



Mex = multiple efflux

MexAB-OprM
MexCD-OprJ
MexEF-OprN
MexXY
MexJK
MexGHI-OpmD
MexVW
MexPQ-OpmE
MexNY
TriABC

FIG. 5. Structure and function of RND efflux pumps in *P. aeruginosa*. RND pumps typically exist in a tripartite system consisting of an RND cytoplasmic membrane transporter (RND), an MFP, and an OMF. This complex forms a channel spanning the entire membrane, allowing for the proton-driven transport of lipophilic and amphiphilic drugs from the cytoplasm of the cell across the cytoplasmic membrane, through the periplasmic space, across the peptidoglycan, and across the outer membrane. The RND efflux pumps can also extrude drugs from the periplasmic space before they cross the cytoplasmic membrane.

Clin Microbiol Rev. 2009 Oct;22(4):582-610

Quinolones resistance Mechanisms

- Alteration of target site (Most common)
  - DNA gyrase (topoisomerasae II)
    - Mutation in quinolone resistance-determining regions (QRDR) of ***gyrA***, *gyrB* genes
  - Topoisomerase IV
    - Mutation in QRDR of *parC*, *parE* genes
- Less common mechanisms
  - Enzymatic inactivation (aac(6')-1b-cr)
  - Decrease permeability
  - Efflux pump (qepA, OqxAB, Mex)
  - Protection of target site

Protection of DNA gyrase and Topoisomerase IV

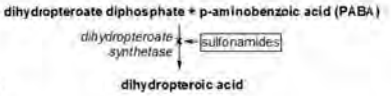
- ***qnr*** gene encoded protein
  - pentapeptide repeat family
  - Plasmid mediated quinolone resistance
- Low-level resistance
- Qnr bind to the DNA gyrase antibiotic target and protect it from quinolone action
- Qnr interact with topoisomerase–quinolone complexes after drug binding and promote release of the quinolone

Daptomycin resistance Mechanism

- Alteration of **cell membrane binding site**
  - Mutation of the gene ***mprF*** (multiple peptide resistance factor)
    - Gene encoding **lysylphosphatidylglycerol synthetase**
- Alteration of cell membrane **phospholipid metabolism**
  - Mutation of the gene ***LiaF*** (in enterococcoci)
    - Involve in cell envelope response to antibiotic

Sulfonamide resistance Mechanisms

- Alteration of target enzyme
  - Dihydropteroate synthetase (DHPS)
  - *sul1* and *sul2* genes -> altered form of enzyme
    - Working enzyme but no longer bind to sulfa
    - *S. maltophilia, E. coli, S pneumoniae*
- Overproduce target enzyme
  - *folP* gene -> normal DHPS
    - Mutations in promotor regions
- Bypass of inhibited process



dihydropteroate diphosphate + p-aminobenzoic acid (PABA)

dihydropteroate synthetase x ← sulfonamides

dihydropteroic acid

↓

dihydrofolic acid

dihydrofolate reductase x ← trimethoprim

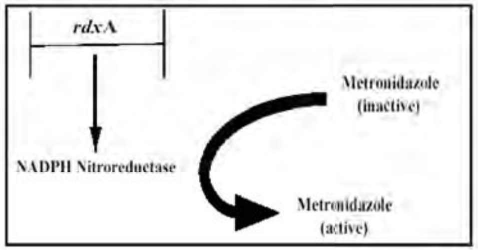
tetrahydrofolic acid

Trimethoprim resistance Mechanisms

- Alteration of target enzyme
  - dihydrofolate reductase (DHFR)
  - *dfrA* gene -> altered form of enzyme
    - Working enzyme but no longer bind to trimethoprim
    - GNB, *Staphylococcus*
- Overproduce target enzyme
  - *folA* gene -> normal DHFR
    - Mutations in promotor regions

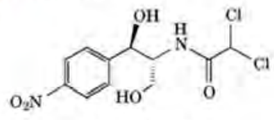
Metronidazole resistance Mechanism

- **Mutation of *rdxA*** gene
  - Chromosomal gene
  - The gene is responsible for production of **"NADPH nitroreductase"**
    - Enzyme that activate metronidazole

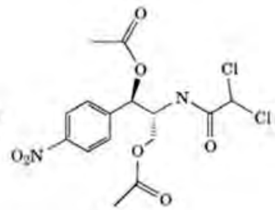


Chloramphenicol resistance

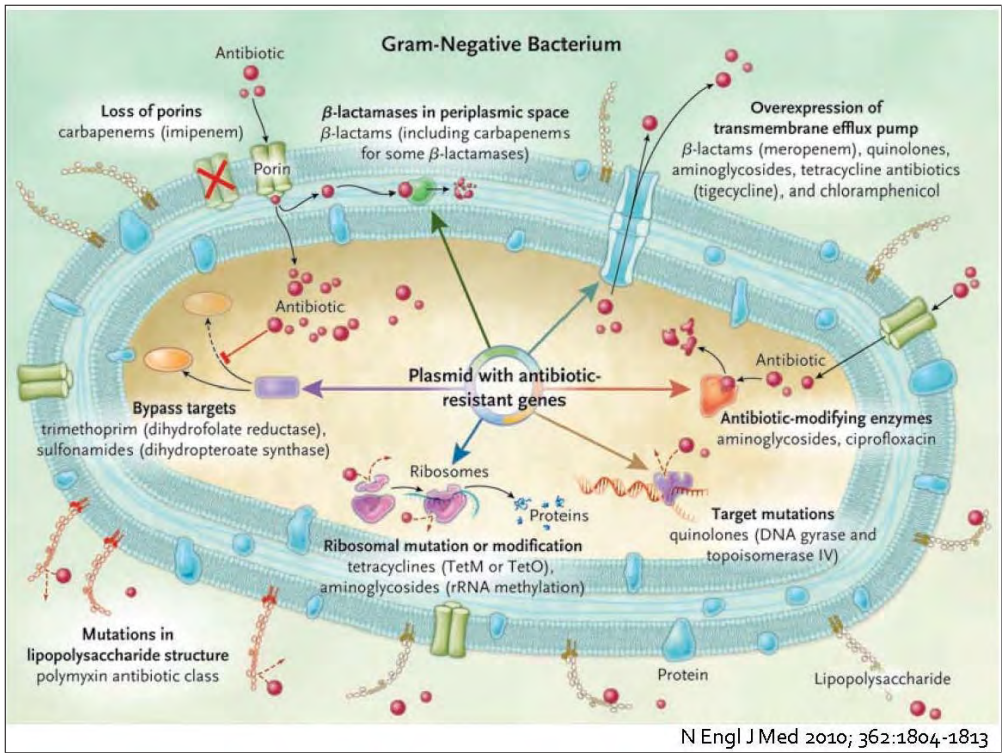
- **Enzymatic inactivation (most common)**
  - **Chloramphenicol acetyltransferase**
    - **3-O-acetylation**
      - **1,3-diacetyl chloramphenicol** (final product)
    - Plasmid or chromosomal gene
      - Encoded by the ***cat*** gene
- Efflux
  - **SmeDEF** Multidrug Efflux Pump
    - RND (Resistance-Nodulation-Division) family
    - Intrinsic in ***Stenotrophomonas maltophilia***
      - Chloramphenicol, tetracycline, erythromycin
      - norfloxacin, ofloxacin
- Decrease permeability
  - Plasmid-mediated in *E. coli*



Chloramphenicol



1,3-diacetyl chloramphenicol



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