

# Understanding Antimicrobial Resistance Mechanisms

Rongpong Plongla, MD MSc D(ABMM)

Division of Infectious Diseases, Department of Medicine  
Faculty of Medicine, Chulalongkorn University  
King Chulalongkorn Memorial Hospital, Thai Red Cross Society  
Rongpong.P@chula.ac.th



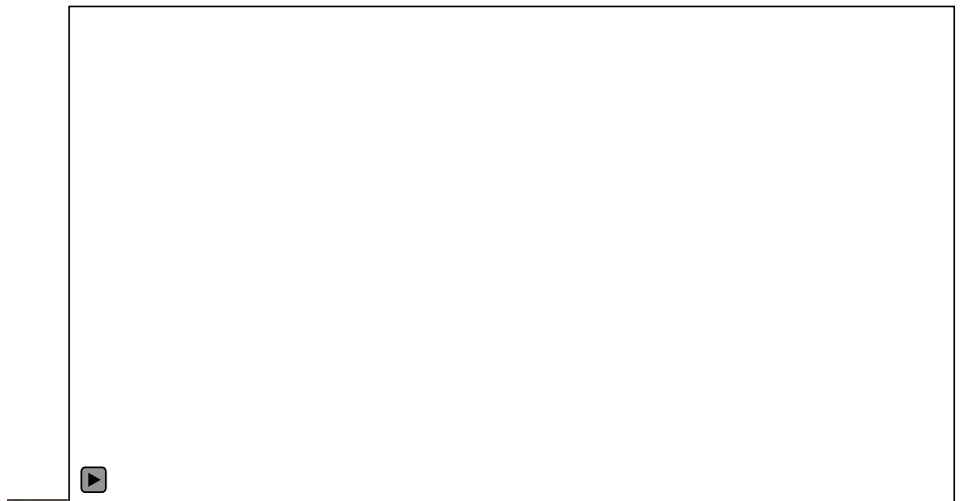
## Outline

- Principle of antibiotic resistance
- Horizontal gene transfers of virulence factors
- Maintaining of antibiotics resistance
- Overview of mechanisms of antibiotic resistance

## Principles of antibiotic resistance

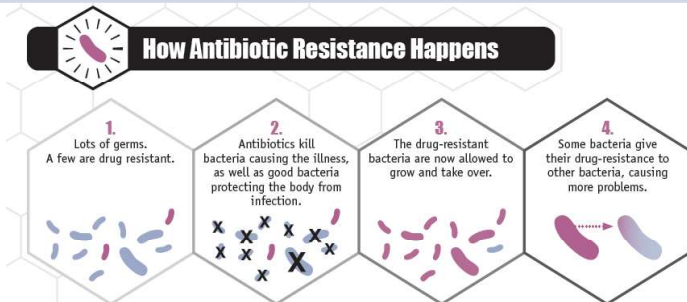
1. Given sufficient time and drug use, antibiotic resistance will emerge
2. Resistance is progressive, evolving from low levels through intermediate and high levels
3. Organisms resistant to one antibiotics are likely to become resistant to other antibiotics
4. Once resistant appears, it is likely to decline slowly, if at all
5. The use of antibiotics by any one person affects others in the extended as well as the immediate environment

## The Evolution of Bacteria on a “Mega-Plate” Petri Dish



Baym M, Lieberman TD, Kelsic ED, Chait R, Gross R, Yelin I, Kishony R. Spatiotemporal microbial evolution on antibiotics landscapes. Science 2016 Sep 9;353:1147-51. <https://www.youtube.com/watch?v=plV4k4NVIU8>

### How Antibiotic Resistance Happens

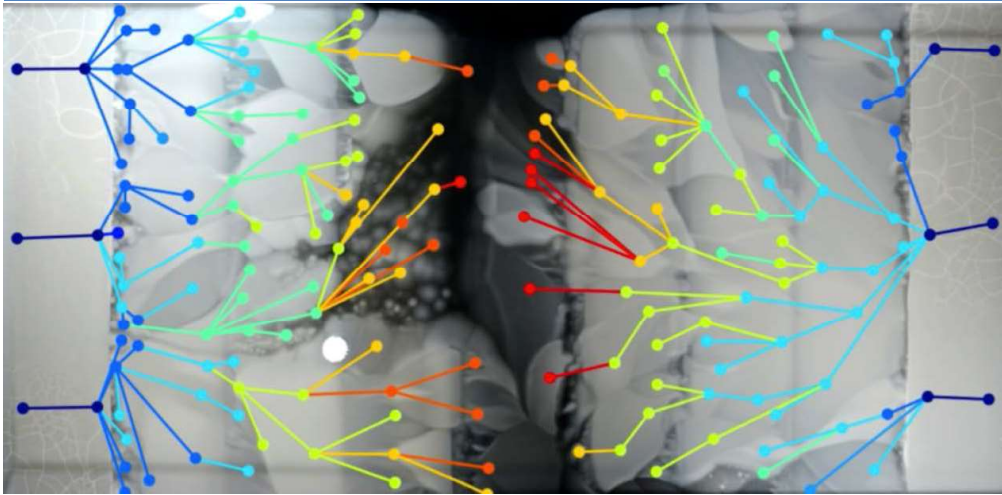


‘SELECTIVE PRESSURE’



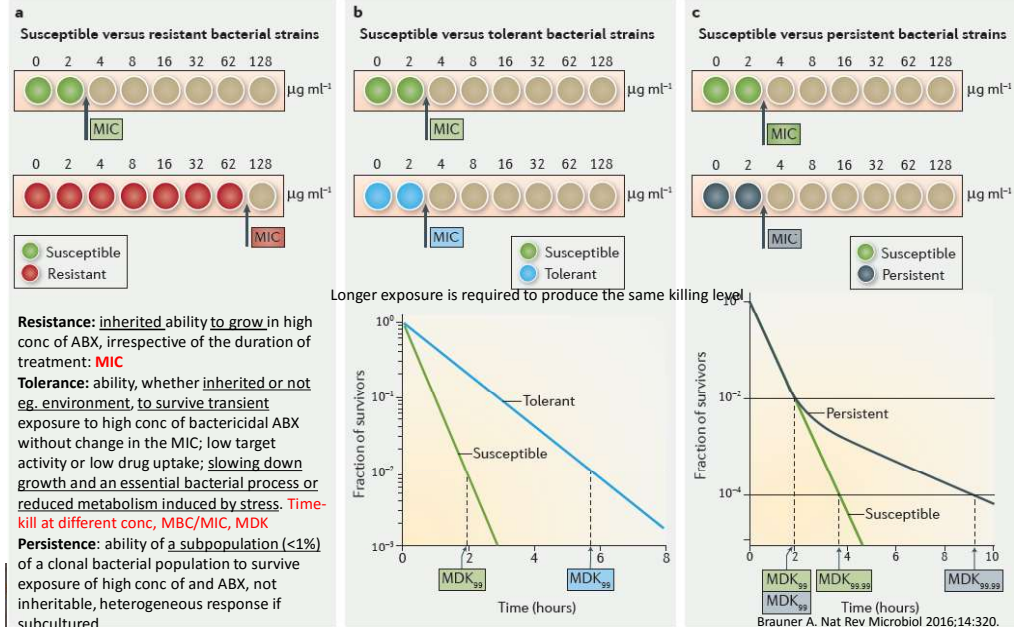
Levy SB. NEJM, 1998  
CDC. Antibiotic Resistance Threats in the United States, 2013

# The Evolution of Bacteria on a “Mega-Plate” Petri Dish



Baym M., Lieberman TD, Kelsic ED, Chait R, Gross R, Yelin I, Kishony R. Spatiotemporal microbial evolution on antibiotics landscapes. Science 2016 Sep 9;353:1147-51. <https://www.youtube.com/watch?v=pIVk4NVIUh8>

# Resistance, Tolerance and Persistence



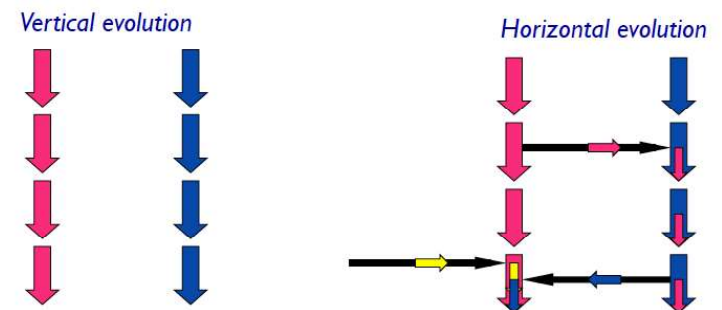
## Mechanisms of genetic change and diversification

Slow process	Rapid process
<ul style="list-style-type: none"> <li>Point mutation: nucleotide change/insertion/deletion: <math>10^{-6}</math>-<math>10^{-9}</math> per nucleotide per generation</li> <li>Gene duplication/ deletion</li> <li>Chromosomal rearrangement: inversion, intragenic recombination</li> </ul>	<ul style="list-style-type: none"> <li>Phase variation: promoter inversion, slipped-strand</li> <li>Antigenic variation: gene shuffling, gene conversion</li> <li>Horizontal gene transfer (HGT)</li> </ul>
e.g. Target or regulator mutation	
Stressed bacteria: hypermutable (error-prone DNA polymerase)	Resistance easily spreads to other bacteria
The bacteria can spread, but resistance does not spread to other bacteria	No apparent fitness cost to the bacteria
Mutations may confer a fitness cost, keeping the rate of resistance low	
Expensive, usually required repeated selection at sublethal dose	

Patel JA. Presentation for CLSI Sept 2016

## Horizontal Gene Transfer (HGT)

- Horizontal (or lateral) gene transfer: any transfer, exchange or acquisition of genetic material that differs from the normal mode of transmission from parents to offspring (vertical transmission)
- Can occur by several mechanisms that cause the transfer/acquisition of genes within a genome, among members of the same species, or between members of very different taxa



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ปี 2560 ครั้งที่ 43

# Mechanisms of Horizontal Gene Transfer (HGT)

- General features: unidirectional (donor to recipient), donor does not give an entire chromosomal, can occur between species

1) **Transformation**: take up free DNA from their surroundings

2) **Transduction**: genes can be moved from one prokaryote species to another via viruses (bacteriophages)

3) **Conjugation**: (bacterial sex) an organism builds a tube-like structure (sex pilus), joins it to its 'mates' and transfers a plasmid through the tube. *E.coli* has been shown to conjugate with cyanobacteria, and even *S. cerevisiae*

## Consequences of HGT

- Gene transfer within species and between species: **no clear species barrier**
  - Conjugation is the most common mode of acquire resistance gene
  - Transformation and transduction: narrow host range (same or closely-related species)
    - > Transformation usually require homozygous recombination for genome integration
    - > Transduction needs bacteria right phage receptor on their surface
- Genome appears as **mosaic**
- Genome are highly **dynamic** in structure

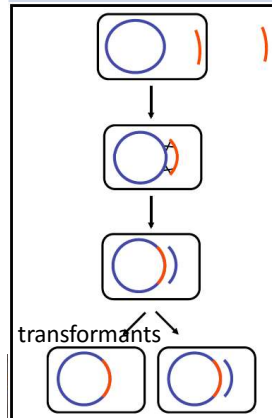


# Transformation

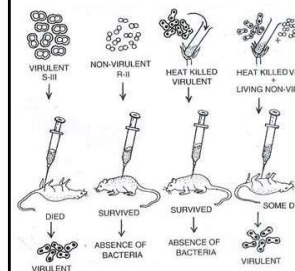
- Gene transfer resulting from the uptake of DNA from a donor
- Recombination: legitimate (homologous or general) and *recA*, *recBC* gene

## Factor affecting transformation

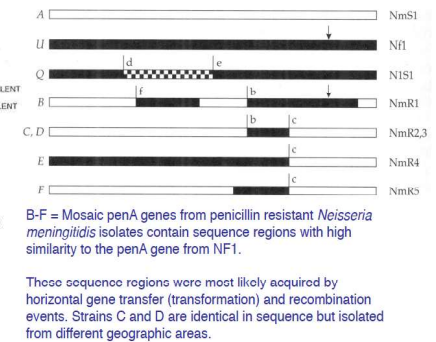
- DNA size and state: sensitive to nucleases
- Competent of the recipient (*Bacillus*, *Haemophilus*, *Neisseria*, *Streptococcus*)



Frederik Griffith's natural transformation

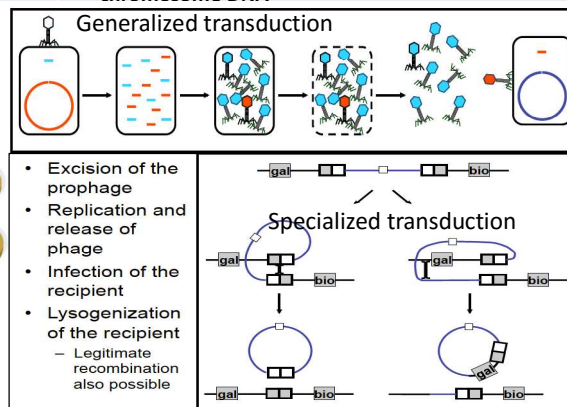
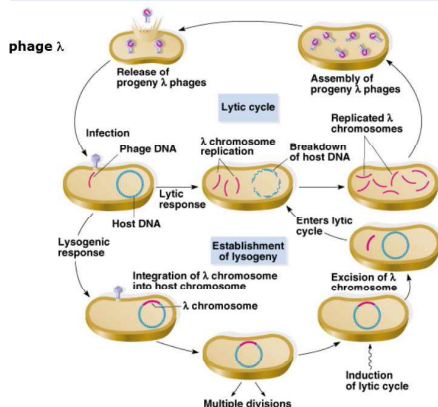


## Penicillin resistant *Neisseria* and *S. pneumoniae*



# Transduction

- Gene (usually 20-100 kb) transfers by a way of bacteriophage
- Lytic (virulent) phage**: multiply within the host cell, lyse the cell and release progeny phage
- Lysogenic (temperate) phage**: can either multiply via the lytic cycle or enter a quiescent state in the bacterial cell
- Resistance to environmental nucleases
- Generalized transduction**: transfers any gene
- Specialized transduction**: transfers specific gene
- Phages typically carry small amounts of DNA, ~1% of host chromosome
- Viral DNA recombines with homologous bacterial chromosome DNA



# Virulence Factors Carried by Phage: Lysogenic conversion

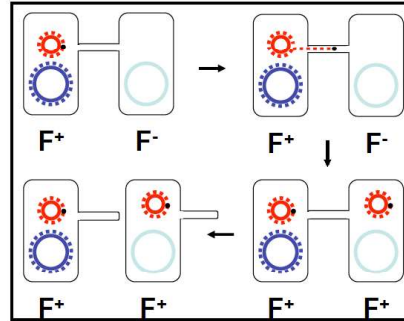
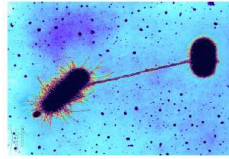
Bacterium	Phage	Gene product	Phenotype
<i>V. cholerae</i>	CTX phage	Cholerae toxin	Cholera
<i>E. coli</i>	Lambda phage	Shiga-like toxin	Hemorrhagic diarrhea
<i>C. botulinum</i>	Clostridial phages	Botulinum toxin	Botulism
<i>C. diphtheriae</i>	Corynephage beta	Diphtheria toxin	Diphtheria
<i>S. pyogenes</i>	T12	Erythrogenic toxin	Scarlet fever





# Conjugation

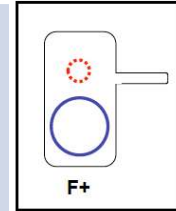
- Transfer of genetic material between donor and recipient cells by direct physical contact
- Segment (rarely all) of donor's chromosome recombines with the homologous recipient chromosome; recipients containing donor DNA are called **transconjugants**
- F (Fertility) factor**: a self-replicating, circular DNA plasmid (1/40 size of the main chromosome) contains an origin sequence (*O*), which initiates DNA transfers.. Also contains gene for *F*-pili (sex pili)
- Conjugation begins when the *F* plasmid is nicked at the origin (*oriT*), and a single strand is transferred using rolling circle mechanism (5' end is translocated to another cell)
- Conjugative plasmid (*oriT*+*tra*), mobilizable plasmid (*oriT*)



# Physiological States of *F* factor

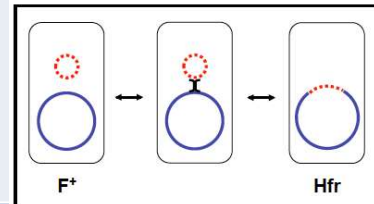
## Autonomous ( $F^+$ )

- Characteristics of  $F^+ \times F^-$  crosses
- $F^-$  becomes  $F^+$ ,  $F^+$  remains  $F^+$
- Low frequency of transfer of donor chromosomal genes



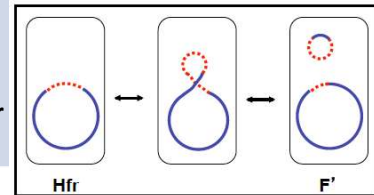
## Integrated F (Hfr)

- Characteristics of Hfr  $\times F^-$  crosses
- $F^-$  rarely becomes Hfr, Hfr remains Hfr
- Highly transfer of certain donor chromosomal genes



## Autonomous F with donor genes ( $F'$ )

- Characteristics of  $F' \times F^-$  crosses
- $F^-$  becomes  $F'$ ,  $F'$  remains  $F'$
- High transfer of donor genes on  $F'$  and low transfer of other donor chromosomal genes



# Transposable Genetic Elements

- Segments of DNA that are able to move from one location to another: insertion sequence, transposon

## Properties:

- random movement
- Not capable of self-replication

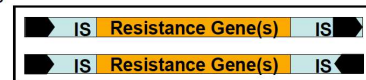
- Transposition mediated by site-specific recombination (independently of homologous recombination); Inverted repeats, Transposases: enzyme performing recombination

Conjugative transposon: can transfer themselves and integrated into the genome (carry *tra* gene that promote transfer + genes promote integrate), transfer intermediate is a covalently closed circle that does not replicate but transfer similarly to a plasmid

- Insertion sequences (IS)
  - Definition: Elements that carry no other genes except those involved in transposition
  - Nomenclature - IS1
  - Structure

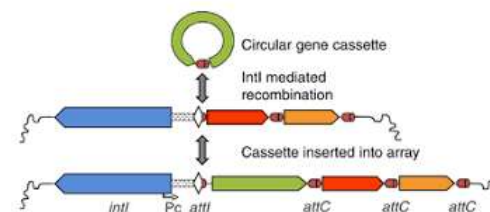


- Transposons (Tn)
  - Definition: Elements that carry other genes except those involved in transposition
  - Nomenclature - Tn10
  - Structure
    - Composite Tn



# Integron

- Integrating element
- Cannot promote self-transfer
- Contain an integrase gene and an attachment (*att*) site
- Integrase integrates circular DNA segment containing a promoterless single open reading frame (gene cassettes) into the *att* site → create operons by sequential integration of the gene cassettes and the *att* site is a promoter provided by the integron
- "Site-specific recombination system capable of recruiting ORF in the form of mobile gene cassettes"

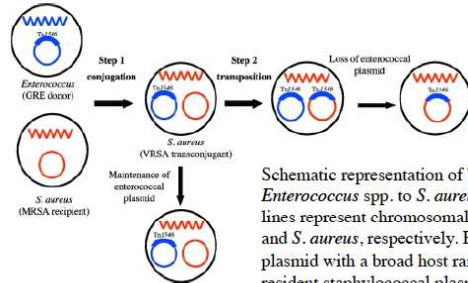


# Conjugation-Transposition: VRSA

- Vancomycin resistance is encoded on conjugative transposons (e.g. Tn1546)
- This transposon is present in many *E. faecalis* strains

The strains harbor a plasmid-borne Tn1546 element following conjugation from a glycopeptide-resistant Enterococcus strain.

In the second step, Tn1546 transposed to a resident plasmid in at least five of the strains; the acquired plasmid behaved as a suicide gene delivery vector, and the incoming DNA had been rescued by illegitimate recombination.

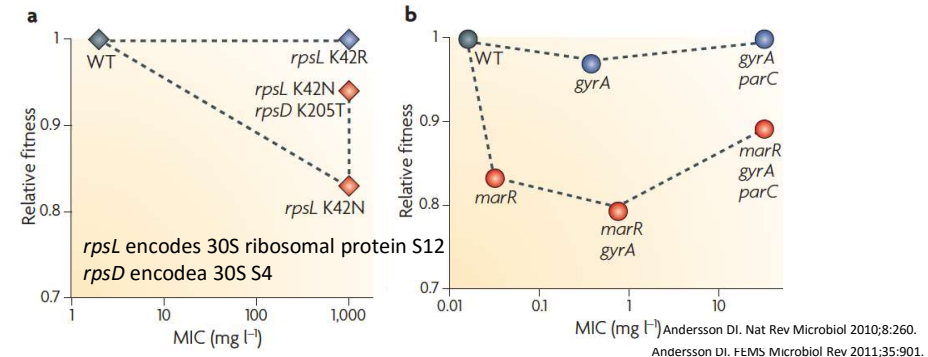


Schematic representation of Tn1546 transfer from *Enterococcus* spp. to *S. aureus*. Blue and red wavy lines represent chromosomal DNA of *Enterococcus* and *S. aureus*, respectively. Blue circle, enterococcal plasmid with a broad host range of transfer; red circle, resident staphylococcal plasmid. Acquisition of Tn1546 was obtained in one step by VRSA-3, -5, and -6 and in two steps by VRSA-1, -7, -8, -9, and -10. GRE, glycopeptide-resistant enterococci.

Antimicrob Agents Chemother. 2009 53:4580-7.  
VanA-type vancomycin-resistant Staphylococcus aureus.  
Perichon B, Courvalin P.

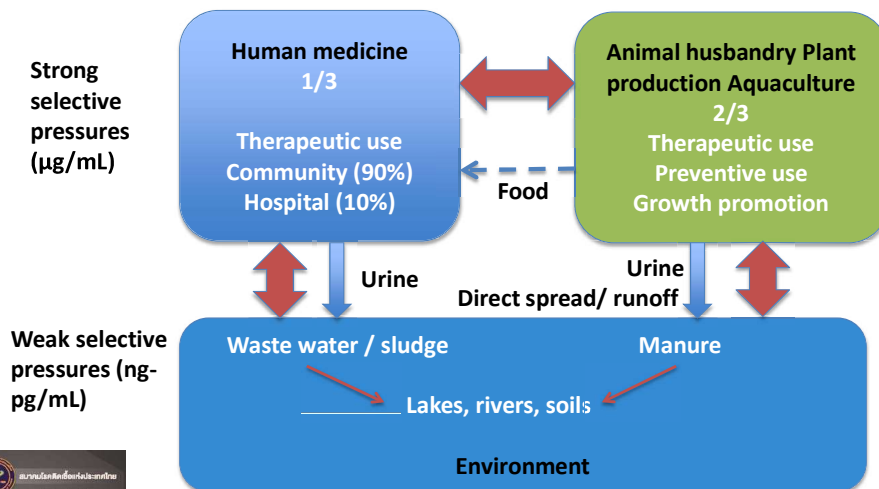
# Maintaining Antibiotic Resistance

- Removing the selective pressure can eliminate antibiotic resistance?
- 1) **Sub MIC selection** (minimal selective concentration)
  - 2) **Regulations of resistance gene**: repressor, translational attenuation, transcription activator
  - 3) **Compensatory mutations** in the same genes (intragenic) or in the other genes (intergenic)
  - 4) **Co-selection (cross-selection)** with between resistance markers/ virulence
  - 5) **Plasmid-mediated resistance**
  - 6) **Cost-free or fitness beneficial mutation** (rare)



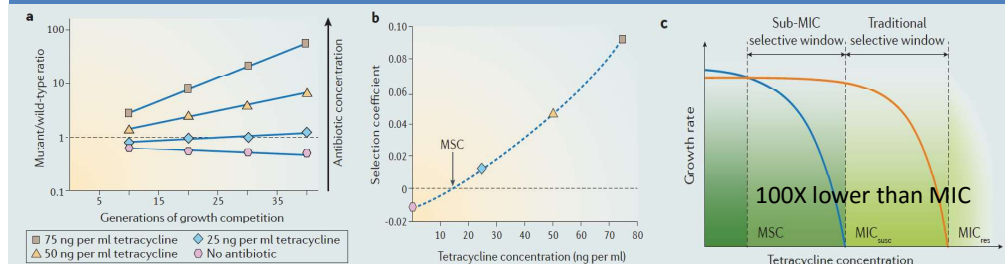
# Where are antibiotic resistant bacteria selected ?

- Globally we use at least 500,000 tons of antibiotics / year

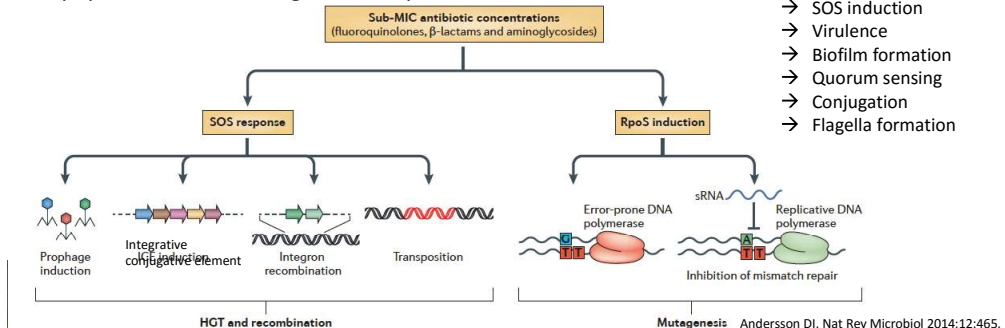


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# Sub MIC Selection



MSC: the lowest concentration of an antibiotic that results in the selection of a resistant mutant in a population over an isogenic susceptible strain



# Resistance

## Intrinsic resistance

- Inherent resistance which is present in nearly all of a particular species
- Susceptibility testing not needed

> vancomycin cannot cross the outer membrane of Gram negative, Daptomycin to Gram negative (has lower proportion of anionic phospholipids), Mycoplasma to betalactams (has no cell wall), *Klebsiella* and beta-lactamase

## Acquired resistance

- Mutations (chromosomal) and acquisition of genes/mobile elements
- Variability in susceptibility pattern

Appendix B  
Intrinsic Resistance

CLSIM100S27

Appendix B. (Continued)

B2. Non-Enterobacteriaceae

Antimicrobial Agent	Organism	Amicillin, Amoxicillin	Piperacillin	Ticarcillin	Ampicillin-sulbactam	Amoxicillin-clavulanate	Piperacillin-tazobactam	Cefazolin	Ceftriaxone	Cefepime	Aztreonam	Imipenem	Meropenem	Ertapenem	Polymyxin B Colistin	Aminoglycosides	Tetracyclines	Trimethoprim-sulfamethoxazole	Chloramphenicol	Fosfomycin
<i>Acinetobacter baumannii</i> / <i>Acinetobacter calcoaceticus</i> complex		R																		
<i>Burkholderia cepacia</i> complex		R	R	R	R	R	R	R	R	R	R	R	R	R						
<i>Pseudomonas aeruginosa</i>		R			R	R	R	R	R	R	R	R	R	R						
<i>Stenotrophomonas maltophilia</i>		R	R	R	R	R	R	R	R	R	R	R	R	R						

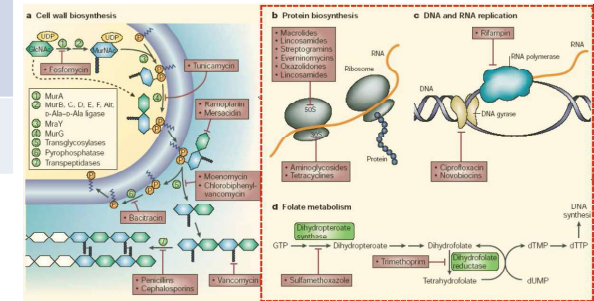
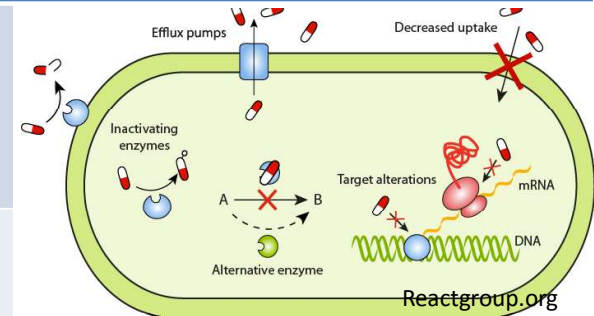
\**Acinetobacter baumannii* may appear to be susceptible to ampicillin-sulbactam due to the activity of sulbactam with this species.  
†*Stenotrophomonas maltophilia* is intrinsically resistant to tetracycline but not to doxycycline, minocycline, or tigecycline.

Thought: All microbial producers of antibiotics must also have ways to protect themselves from ABX-coevolved or preacquired resistance gene



# Mechanisms of resistance

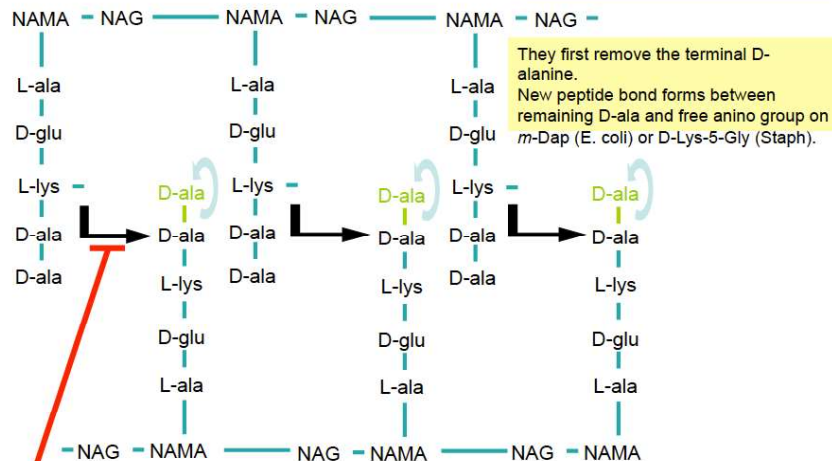
- Target alteration/ bypass  
DNA gyrase: Fluoroquinolones  
Penicillin-binding protein: Penicillin  
Gram positive cell wall: Vancomycin  
Ribosome: Tetracyclines, macrolides  
Metabolic pathway: TMP/SMX  
overproduction of target
- Antibiotic degrading/modifying enzyme  
: Aminoglycosides, beta-lactams
- Decreased membrane permeability  
: Many antibiotics
- Efflux pumps  
: Tetracyclines, macrolides



## Mechanisms of Resistance in Gram-Positive Bacteria

### Peptidoglycan synthesis - Transpeptidation

Linear glycan strands are crosslinked by a group of transpeptidases located on the outer surface of the cytoplasmic membrane.



$\beta$ -lactams antibiotics resemble D-ala-D-ala, bind to transpeptidase (PBP) enzyme, inhibit cross-linking - Cell lyses

Different  $\beta$ -lactams bind different PBP's preferentially

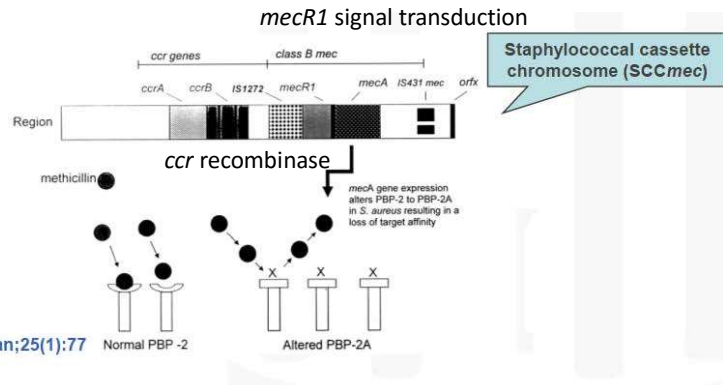
PBP	% of total PBP	Function
1a { 1b { Penicillin G, Ampicillin	8.1	Transpeptidases involved in peptidoglycan synthesis during elongation
2 3 Mecillinam	0.7 1.9	Required for maintenance of "rod" shape Required for septum formation
4 5 6 These bind antibiotic but inhibition is not lethal - they do not catalyze cross-linkage Up to 90% of the antibiotic is consumed here!!	4.0 64.7 20.6	D-alanine carboxypeptidases



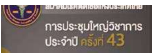


# MRSA/ ORSA

- PBP (penicillin binding protein) 2a: altered PBP encoded by *mecA* that results in resistance to nearly all beta-lactam drugs



Rybak MJ, LaPlante KL  
Pharmacotherapy. 2005 Jan;25(1):77



## VISA

Thickened cell wall: global cell adaptations/ regulatory systems

- Accelerated peptidoglycan synthesis
- Increased proportion of dipeptides capable of binding or trapping vancomycin; vancomycin sponge
- May be lost if vancomycin selective pressure is removed
- Also associated with reduced susceptibility to daptomycin

Vancomycin MIC 4-8 µg/mL

- Cannot reliably detect by disk diffusion
- Add Etest and/or vancomycin screening agar (6 µg/mL)
- Broth microdilution and automated susceptibility test systems should detect

hVISA

- Strains containing subpopulation of VISA cells
- MICs for parents strains will be in the susceptible range
- Not detected by routine MIC or disk diffusion
- Reduced clinical success with vancomycin
- 10-18% of MRSA with vancomycin MIC = 2 µg/mL

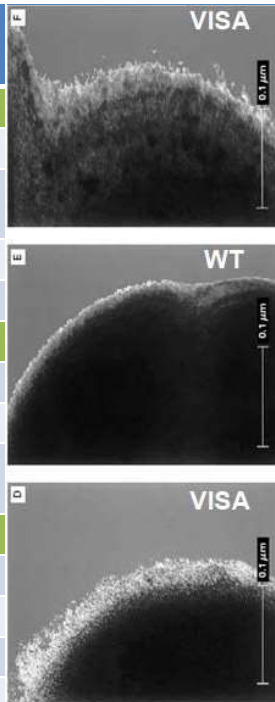


Figure 3. Electron Micrographs of *S. aureus* isolates.

Smith et al., NEJM 1999;340

Howden. CMR2010; 99-139.

# MRSA/ ORSA

## That are not *mecA* mediated

### BORSA

- Mediated by overexpression of *blaZ*

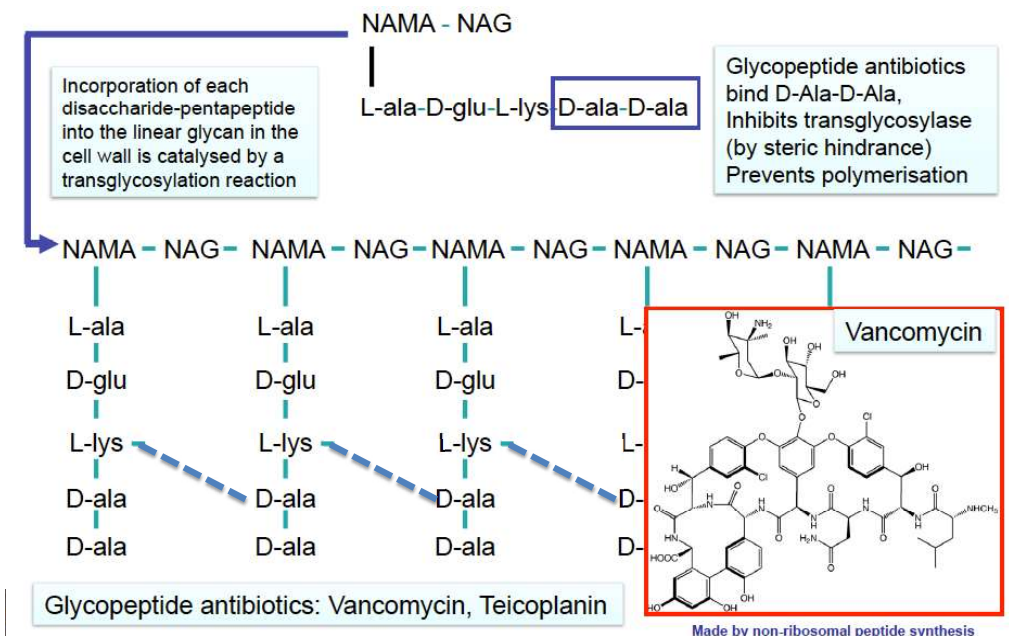
### *mecC*

- *mecA* homologue in human and bovine MRSA; UK, Denmark and Ireland; 70% homology to *mecA*, carry on type XI SCCmec

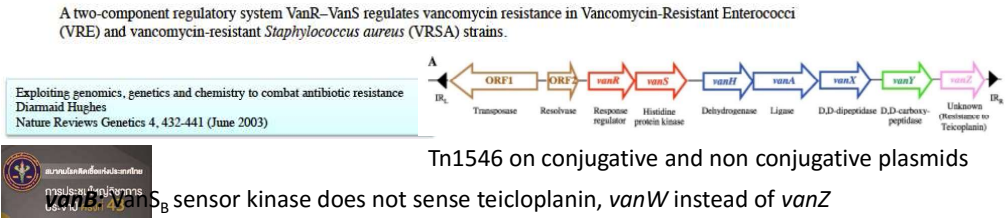
Cefoxitin	Oxacillin	Mechanism	Prevalence	Report as OX:
S	S	None	Common	S
R	R	<i>mecA</i>	Common	R
S	R	<i>mecC</i>	Uncommon	R
S	R	<i>mecA</i> (low level expression)	Uncommon	R
R	S	PBP changes or hyperproduction of beta-lactamase (BORSA)	Rare	R

Laurent. EID 2012;18:1465.  
Garcia-Alvarez. Lancet ID 2011; 11:595.

## Peptidoglycan synthesis - assembly at the growing point



## Vancomycin resistance mechanisms



## Vancomycin and Enterococci

**VRSA (MIC  $\geq 16$   $\mu\text{g/mL}$ ): acquisition of *vanA* from enterococci; rare**

- **Terminal pentapeptide precursor (vancomycin binding site) is modified from D-alanine-D-alanine to D-alanine-D-lactate**

VanA-MV-2D Lac, VanCEGN: D-ser: low-level resistance

## Enterococci

## Penicillin and Ampicillin

- **Beta-lactamase producer (rare): test by nitrocefin**

### Intrinsic resistance

- **Cephalosporins:** PBP5 in *E. faecium* (confers ampicillin and imipenem resistance), PBP4 in *E. faecalis*
- **Clindamycin:** efflux pump (chromosomally Lsa)
- **Trimethoprim-sulfamethoxazole:** absorption of folic acid from the environment

## B-hemolytic streptococci

## Penicillin

- Emergence of elevated MICs to beta-lactams due to **mutation to PBP2x (rare)**
- Kimura. JAC 2015 PMID 25667405

## Clindamycin

- Could be **efflux (*mefA*)** or **inducible *erm* gene**
- Must screen for inducible resistance

## Vancomycin

- **vanG** from *E.faecalis*
- MIC 4 µg/mL

## *Streptococcus pneumoniae*

## Penicillin

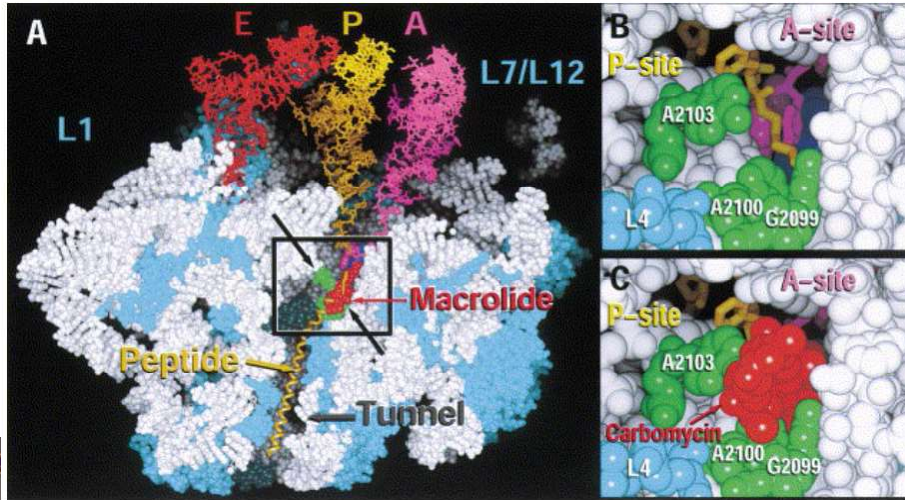
- transformation (recombination with DNA from *S. mitis*)



## Macrolides

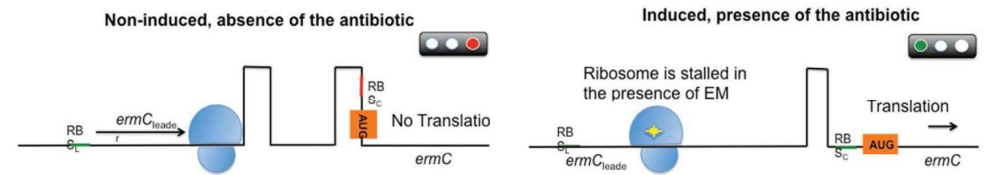
Bind 50S ribosomal subunit and prevent peptide chain elongation by blocking the exit tunnel.

Macrolide antibiotics block protein synthesis by binding to bacterial ribosomes and preventing exit of the peptide from the ribosome.



## Erm (erythromycin ribosomal methylation)

- RNA methylases: ErmA (MRSA transposon), ErmB (Enterococci, pneumococci), ErmC (MRSA plasmid) ErmF, ErmG: **MGE**
- Add 1-2 methyl groups to A2058 adenine in 23S rRNA
- A2058 base form hydrogen bond with antibiotic (macrolides, streptogramins, lincosamide) MLS<sub>B</sub>
- Methylation of A2058 → prevent hydrogen bond → less tightly bind to macrolide
- Translation attenuation: posttranslational gene regulation



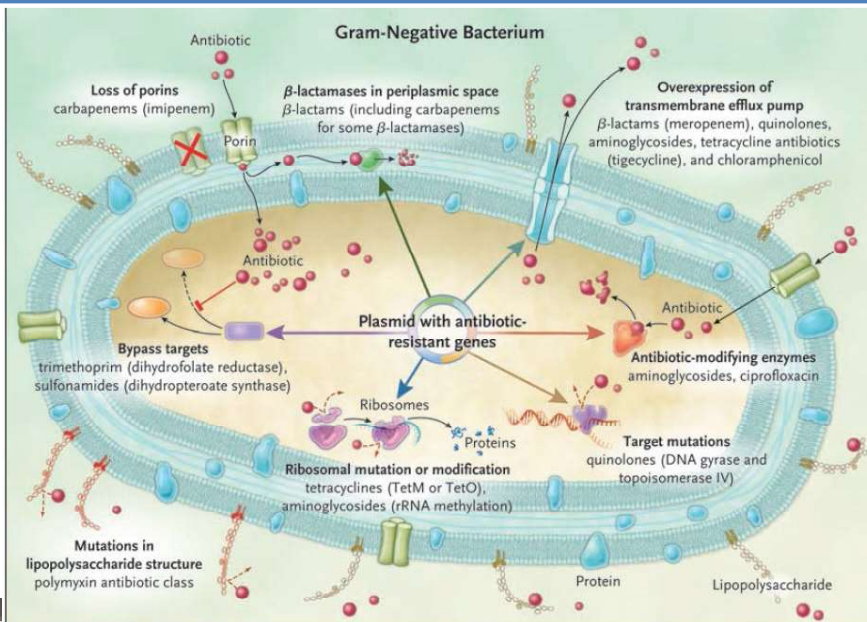
Munita JM. Virulence Mechanisms of bacterial pathogens, 5<sup>th</sup> ed. ASM 2016

## mef

- MefA on Tn1207 in the chromosome, MefE in macrolide efflux genetic assembly (MEGA) element in chromosome: no cross resistance to lincosamide and S<sub>B</sub>

Note: Other methylation: cfr (chloramphenicol-florfenicol resistance) methyltransferase: methylate A2503 in the 23S rRNA: **plasmid** mediated resistance to streptogramins, lincosamide, oxazolidinones

## Mechanisms of Resistance in Gram-Negative Bacteria



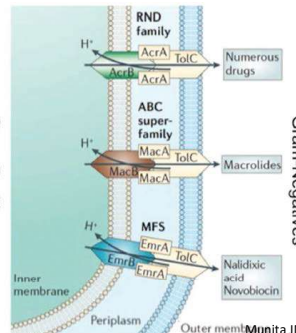
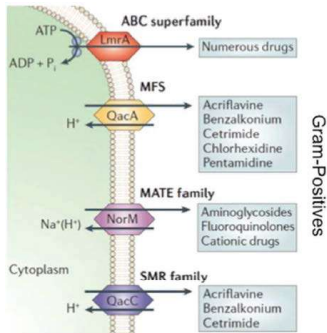
Peleck AY. NEJM 2010; 362:18-4-13

## Outer membrane porins

- Beta-barrel structures that allow selective diffusion of small molecules into the periplasm
- Beta-lactams must transit the gram-negative outer membrane to reach cytoplasmic membrane, where PBPs are located
- Other antibiotics that have targets in bacterial cytoplasm e.g. macrolides
- Vancomycin: too bulky to diffuse through outer membrane porin proteins
- Usually confers increases in resistance of 5- to 10- fold
- Gram-negative bacteria encode many different porins with a variety of permeability limits, and changing stress conditions regulate the expression of porin genes
  - OmpF and OmpC of *E.coli* (non-specific channel), OprD of *P.aeruginosa*
  - *Enterobacteriaceae*, *Acinetobacter*, *Pseudomonas*
  - 1) down regulation (reductions in porin expression)
  - 2) replacement with more selective channels
  - 3) impairment of porin function
  - eg. aberrant production of OprD in PAER, shift in porin expression from OmpK35 to OmpK36 (smaller channel size) in KP

# Active Efflux

- Membrane proteins that use energy to pump small molecules
- Normally play roles in maintaining homeostasis by pumping metabolites and toxic substances
- Some are highly specific (e.g. TetA [MFS]), some pump out many compound (MdeA in *S. mutans*, FuaABC in *S. maltophilia*, KexD in *K. pneumoniae*, LmrS in *S. aureus*)
- Resistance nodulation division (RND) efflux pump: tripartite complex; frequently found on chromosome
  - > AcrAB-TolC in *E. coli*, MexAB-OprM in *P. aeruginosa*
  - > Immobilized onto plasmids (e.g. IncH1 of *C. freundii* that also carried NDM1 gene)
- Increased expression: increased expression of transcription factor (MarA, SoxS, Ram, Rob in *Enterobacteriaceae*, mutation in regulatory network, induction in response to environmental signals e.g. acrAB in *Salmonella-E. coli* responds to indole and bile, MtrCDE of *N. gonorrhoeae* responds to iron limitation)



**MFS:** major facilitator superfamily  
**SMR:** small multidrug resistance family  
**RND:** Resistance nodulation cell division family  
**ABC:** ATP binding cassette family  
**MATE:** multidrug and toxic compound extrusion family  
 > Structure conformation, energy source, range of substrates

Munita JM. Virulence Mechanisms of bacterial pathogens, 5<sup>th</sup> ed. ASM 2016

# $\beta$ -lactamases: Ambler classification

(amino-acid structure)

CLASS	ACTIVE SITE	ENZYME TYPE	SUBSTRATES	EXAMPLE
A	Serine	Penicillinases:		
2	Clavulanate	Broad-spectrum	Benzylpenicillin, aminopenicillins, carboxypenicillins, ureidopenicillins, narrow-spectrum cephalosporins	PC1 in <i>Staphylococcus aureus</i> TEM-1, SHV-1 in <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , other gram-negative bacteria
		Extended-spectrum ( $\beta$ -lactamase)	Substrates of broad-spectrum plus oxymino- $\beta$ -lactams (cefotaxime, ceftazidime, ceftriaxone) and <del>aztreonam</del> <b>Not cephamycins</b>	In <i>Enterobacteriaceae</i> : TEM-derived, SHV-derived, CTX-M-derived; PER-1, VEB-1, VEB-2, GES-1, GES-2, IBC-2 in <i>Pseudomonas aeruginosa</i>
		Carbapenemases	Substrates of extended-spectrum plus cephamycins and carbapenems	KPC-1, KPC-2, KPC-3 in <i>K. pneumoniae</i> : NMC/IMI, SME family
B	3	Metallo- $\beta$ -lactamases ( $Zn^{2+}$ )	EDTA, Aztreonam is poor substrate	NDM-1 in <i>Enterobacteriaceae</i> , IMP, VIM, GIM, SPM, SIM lineages in <i>P. aeruginosa</i> , <i>Acinetobacter</i> spp.
C	1	Serine Boronic acid	Cephalosporinases <b>Cefepime is poor substrate</b>	AmpC-type enzymes in <i>Enterobacteriaceae</i> , <i>Acinetobacter</i> spp.
D	Serine	Oxacillinases:		
2d		Broad-spectrum	Aminopenicillins, ureidopenicillin, cloxacillin, methicillin, oxacillin, and some narrow-spectrum cephalosporins	OXA-family in <i>P. aeruginosa</i>
		Extended-spectrum	Substrates of broad-spectrum plus oxymino- $\beta$ -lactams and monobactams	OXA-derived in <i>P. aeruginosa</i>
		Carbapenemases	Substrates of extended-spectrum plus cephamycins and carbapenems	OXA-derived in <i>Acinetobacter</i> spp.

AmpC, ampicillin C; CTX-M, cefotaxime-M; GES-1, -2; Guyana extended-spectrum  $\beta$ -lactamase-1, -2; GIM, German Imipenemase; IBC-2, integron-born cephalosporinase; IMI, imipenem hydrolyzing; IMP, imipenem; KPC-1, -2, -3, *K. pneumoniae* carbapenemase-1, -2, -3; NDM-1, New Delhi metallo- $\beta$ -lactamase-1; NMC, not metalloenzyme carbapenemase; OXA, oxacillin; PC1, penicillin 1; PER-1, *Pseudomonas* extended resistance-1; SHV-1, sulfhydryl variable-1; SIM, Seoul imipenemase; SME, *Serratia marcescens* extended-spectrum  $\beta$ -lactamase; SHM, Sao Paulo metallo- $\beta$ -lactamase; IEM-1, Iemoneira-1; VEB-1, -2, Vietnam extended-spectrum  $\beta$ -lactamase-1, -2; VIM, Verona integron-encoded metallo- $\beta$ -lactamase.

**Serine class:** forms covalent bonds between active serine residue (same as transpeptidases) and beta-lactam ring  
**MBL:** require one or more divalent cations at the active site for activation

**CTX-M:** escapes from the chromosome of *Kluyvera* (soil) facilitated by IS (insertion sequence) *Ecp1* --> conjugative plasmid  
 KPC: pKP-Qil, **NDM:** broad plasmid types, extremely mobile *ISAbi125*

Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. Elsevier Health Sciences; 2014.

## ampC

- Primarily hydrolyze cepheims (cephalosporins and cephamycins), penicillins and aztreonam, resistance to clavulanate

### Inducible chromosomal ampC gene

- SPI(A)CE: *Serratia*, *Pseudomonas aeruginosa*, Indole-positive *Proteae*, *Acinetobacter*, *Citrobacter*, *Enterobacter cloacae*

### Expression of ampC

- 1) uninduced, produced at low levels: not result in *in vitro* resistance
- 2) induced, upregulation of expression: reversible, not thought to contributed to clinical failure?
- 3) stably derepressed or hyper-produced: a mutation ( $10^{-6}$  to  $10^{-9}$  in the regulatory machinery of this gene: treatment failure with 2<sup>nd</sup>/3<sup>rd</sup> gen ceph

### Inducer potentials of beta-lactams agent

Good	Variable	Poor
<ul style="list-style-type: none"> <li>• Cefoxitin</li> <li>• Cefmetazole</li> <li>• Imipenem</li> <li>• Meropenem</li> <li>• Ampicillin</li> </ul>	<ul style="list-style-type: none"> <li>• Clavulanate</li> <li>• Desacetyl cefotaxime</li> <li>• Cefamandole</li> <li>• Cephalothin</li> <li>• Cefonicid</li> </ul>	<ul style="list-style-type: none"> <li>• Sulbactam</li> <li>• Tazobactam</li> <li>• Aztreonam</li> <li>• Third generation cephalosporins</li> <li>• Fourth generation cephalosporins</li> </ul>

## ampC

### Plasmid-mediated ampC gene

- *E. coli*, *K. pneumoniae*, *P. mirabilis*, *Salmonella*
- Derived from *E. cloacae*, *C. freundii*, *M. morgani*, *H. alvei*
- Most are constitutively expressed
- Some are Inducible: DHA-1, DHA-2, ACT-1, CFE-1, CMY-13



## Quinolones

- Inhibition of DNA replication and synthesis**
  - Target against topoisomerase II (DNA gyrase) and IV**
    - ✗ Introduce negative supercoiling
    - ✗ Decatenation of daughter chromosomes
    - Double-strand breaks
- Gyrase re-ligates DNA**

**How fluoroquinolones work**

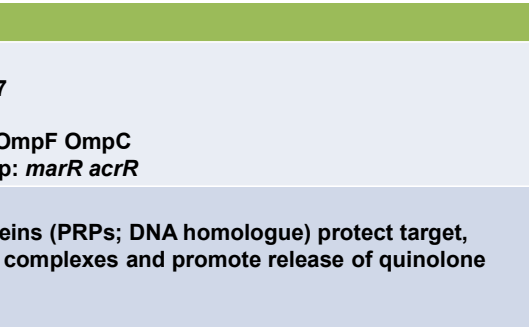
Gyrase cuts DNA

Fluoroquinolone binds gyrase-DNA complex

Fluoroquinolone prevents Gyrase re-ligation of DNA

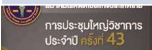
Chromosome acquires double-strand break

The diagram illustrates the mechanism of fluoroquinolones. It shows a DNA double helix with a gyrase complex (orange) bound to it. A fluoroquinolone molecule (red) is shown binding to the gyrase-DNA complex. This leads to 'Chromosome fragmentation (protein synthesis independent)' and 'Flapid cell death'. A dashed line indicates 'SDS-dependent release of DNA breaks after cell lysis'.



## Mechanisms of resistance

- Chromosomal-mediated
  - Target mutation: QRDRs *gyrA* S83 D87
  - Decrease drug accumulation
    - Outer membrane porin proteins: OmpF OmpC
    - ArcAB-TolC multidrug efflux pump: *marR acrR*
- Plasmid-mediated
  - Qnr: encode pentapeptide repeat proteins (PRPs; DNA homologue) protect target, interact with topoisomerase-quinolone complexes and promote release of quinolone
  - AAC(6')-Ib-cr: modifying enzyme
  - Efflux pumps: QepA OqxAB



## Polymyxin

- **Lipid A modification**
- **Membrane permeability**
  - Chromosomal mutations: *mgrB*, overexpression of *pmrC*, mutations in the gene encoding the PhoPQ component system or its regulator → increased expression of PmrAB system
  - Plasmid-mediated resistance: *mcr-1* Phosphoethanolamine transferase



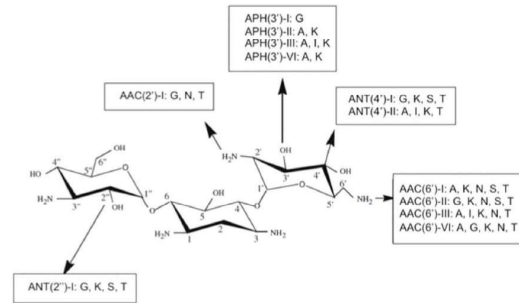
## Aminoglycoside

Large molecules with many exposed hydroxyl and amide groups

## Aminoglycoside-modifying enzymes

- Adding phosphoryl (APH), adenylyl/nucleotidyl (ANT), or acetyl (AAC) groups to

- Interfere with the hydrogen bonding network that antibiotics use to bind tightly to 16S rRNA and to inhibit translation
- Usually harbored in MGEs, part of chromosome if *P. stuartii*, *E. faecium*, *S. marcescens*

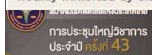


**Methyltransferases: *armA*, *rmt***

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## Summary: Mechanisms of Resistance in Gram-Negative Bacteria

Structure and function	Genetics	Common species	Common examples
<b>Production of class A* <math>\beta</math>-lactamases<sup>†</sup></b>			
Contain serine residues Key features of ESBLs are cephalosporinase activity and resistance to 3GCs Usually inhibited by clavulanate-tazobactam <i>in vitro</i> (except KPC)	ESBLs usually arise from mutations in 'parent' narrow-spectrum $\beta$ -lactamase or have been 'captured' from environmental bacteria (e.g. CTX-M from <i>Kluyvera</i> spp.) Transmissible on mobile genetic elements, such as plasmids, carrying multiple other resistance determinants	ESBLs are most common in <i>Escherichia coli</i> , <i>Klebsiella</i> spp. and <i>Proteus</i> spp., but have been described in most Enterobacteriaceae and in <i>Pseudomonas</i> spp. KPC is seen in <i>K. pneumoniae</i>	ESBLs: TEM and SHV variants, CTX-M Carbapenemase: KPC
<b>Production of class B* <math>\beta</math>-lactamases<sup>†</sup></b>			
Contain metal ions (for example, Zn <sup>2+</sup> ) Have carbapenemase activity, not inhibited by clavulanate/tazobactam Aztreonam not hydrolysed by class B $\beta$ -lactamases	Usually highly transmissible on plasmids carrying multiple other resistance determinants	<i>E. coli</i> , <i>Klebsiella</i> spp. but described in many Enterobacteriaceae and <i>Acinetobacter</i> spp. Intrinsic carbapenem resistance in <i>Stenotrophomonas maltophilia</i> via class B enzyme (L-1)	Carbapenemase: IMP, NDM, VIM
<b>Production of class C* <math>\beta</math>-lactamases<sup>†</sup></b>			
Also known as 'AmpC' enzymes Broad cephalosporinase activity including 3GCs, but cefepime stable Not inhibited effectively by clavulanate or tazobactam	Chromosomally encoded in many species, but can also be inducible in some Enterobacteriaceae Mutations in regulatory genes (for example, <i>ampD</i> or <i>ampR</i> ) involved in cell-wall recycling can lead to high-level AmpC expression and resistance to a broad range of $\beta$ -lactams Increasing plasmid-AmpC transmission described	<i>Enterobacter cloacae</i> , <i>E. aerogenes</i> , <i>Serratia marcescens</i> , <i>Citrobacter freundii</i> , <i>Pseudomonas aeruginosa</i> , <i>Providencia</i> spp. and <i>Morganella morganii</i> all contain inducible AmpC enzymes that are chromosomally encoded Plasmid-mediated AmpC (e.g. CMY) increasing in <i>E. coli</i>	Cephalosporinase: CMY, DHA, ACT
<b>Production of class D* <math>\beta</math>-lactamases<sup>†</sup></b>			
Oxacillinases that can have carbapenemase activity and are only weakly inhibited by clavulanate	Can be acquired or naturally occurring chromosomal genes	<i>Acinetobacter baumannii</i> (e.g. OXA-23), Enterobacteriaceae (e.g. OXA-48), <i>Pseudomonas</i> spp.	Carbapenemase: OXA-type





Summary:  
Mechanisms of Resistance in Gram-Negative Bacteria

Efflux pumps and porin mutations			
Membrane transport systems to extrude multiple antimicrobials or mutations in outer membrane proteins to hinder entry of active drug	Poly-specific transporters; for example, the resistance-nodulation-division (RND) family Porin changes or loss via mutation	<i>Pseudomonas aeruginosa</i> , <i>Acinetobacter baumannii</i> , Enterobacteriaceae (e.g. <i>Enterobacter</i> spp.)	Efflux: AcrAB-like or MexAB-OprM Porin: OprD channel loss
Target site mutations			
Methylation of 16S rRNA with high-level resistance, including against amikacin	Carried by plasmids, often mediated by <i>rmtA</i> and related genes	Enterobacteriaceae (e.g. <i>K. pneumoniae</i> )	Methylases: RmtA, RmtB or ArmA
Altered DHPS—essential for folate synthesis in bacteria—leads to sulphonamide resistance and altered DHFR with loss of inhibition by trimethoprim	<i>sul1</i> gene part of class 1 integron—frequently integrated into plasmids Plasmid-borne <i>dhfrI</i> and <i>dhfrII</i> genes	<i>Stenotrophomonas maltophilia</i> , <i>E. coli</i>	<i>sul1</i> and <i>dhfr</i> genes
DNA gyrase mutations prevent activity of quinolones	Often single mutations in quinolone resistance-determining region (QRDR) on <i>gyrA</i> gene	<i>Pseudomonas aeruginosa</i> , <i>Salmonella</i> spp., other Enterobacteriaceae	Point mutations in <i>gyrA</i> , <i>gyrB</i> , <i>parC</i> and <i>parE</i> genes
Protein binding of quinolone active site, low-level resistance	Protein binding encoded by plasmid-mediated <i>qnr</i> genes	<i>E. coli</i> , <i>Klebsiella</i> spp., <i>Salmonella</i> spp.	<i>qnrA</i> , <i>qnrB</i> , <i>qnrC</i> , <i>qnrD</i> and <i>qnrS</i>
Overproduction of enzymes			
Sulphonamide or trimethoprim resistance by overproduction of DHPS or DHFR	<i>feIP</i> gene produces DHPS and <i>folA</i> encodes DHFR	<i>E. coli</i>	Mutations in promotor regions result in increased production of DHFR and trimethoprim resistance
Drug modification			
Acetylation, nucleotidylation or phosphorylation of aminoglycosides Some can also inactivate fluoroquinolones	Often carried on transmissible elements such as plasmids or transposons Can be chromosomal in some species	<i>Pseudomonas aeruginosa</i> and Enterobacteriaceae Intrinsic to <i>Providencia</i> spp.	AAC(2''), ANT(2''), APH(2'') AAC(6'')-Ib-cr (also confers quinolone resistance)

Summary

