Antimicrobial Resistance: A Broad-Spectrum Health Crisis

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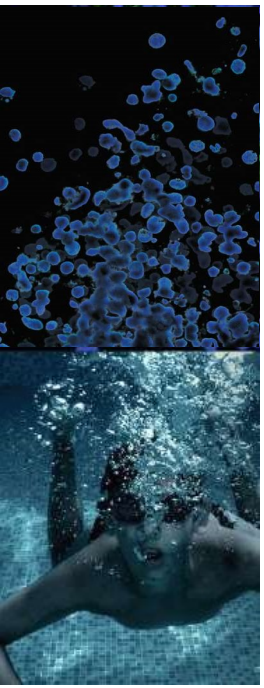
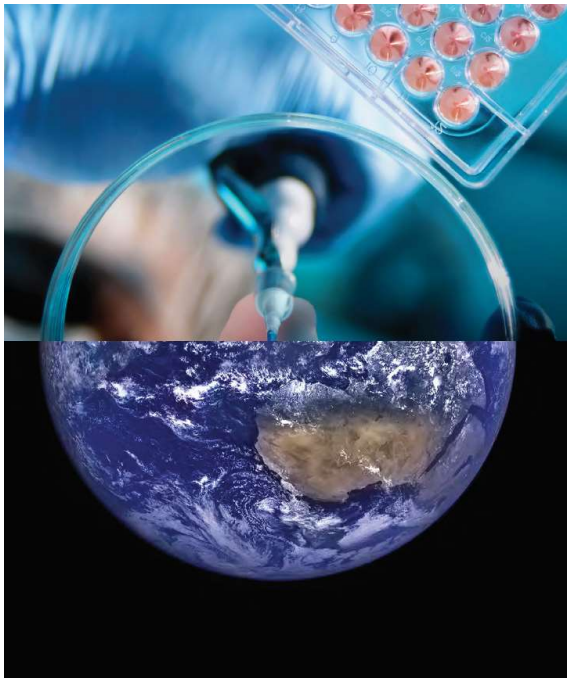
Microbiologist, ATCC

Credible Leads to Incredible™





About ATCC

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's largest, most diverse biological materials and information resource for microbes – the "*gold standard*"
- Innovative R&D company featuring gene editing, microbiome, NGS, advanced models
- cGMP biorepository
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees

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Overview

Introduction:

- What antimicrobial resistance is

History:

- Antibiotics and resistance in history
- Key events in modern medicine
- Current state of affairs

Mechanisms:

- How antibiotics work
- How resistance works
- How resistance spreads

Summary:

- Urgent threats

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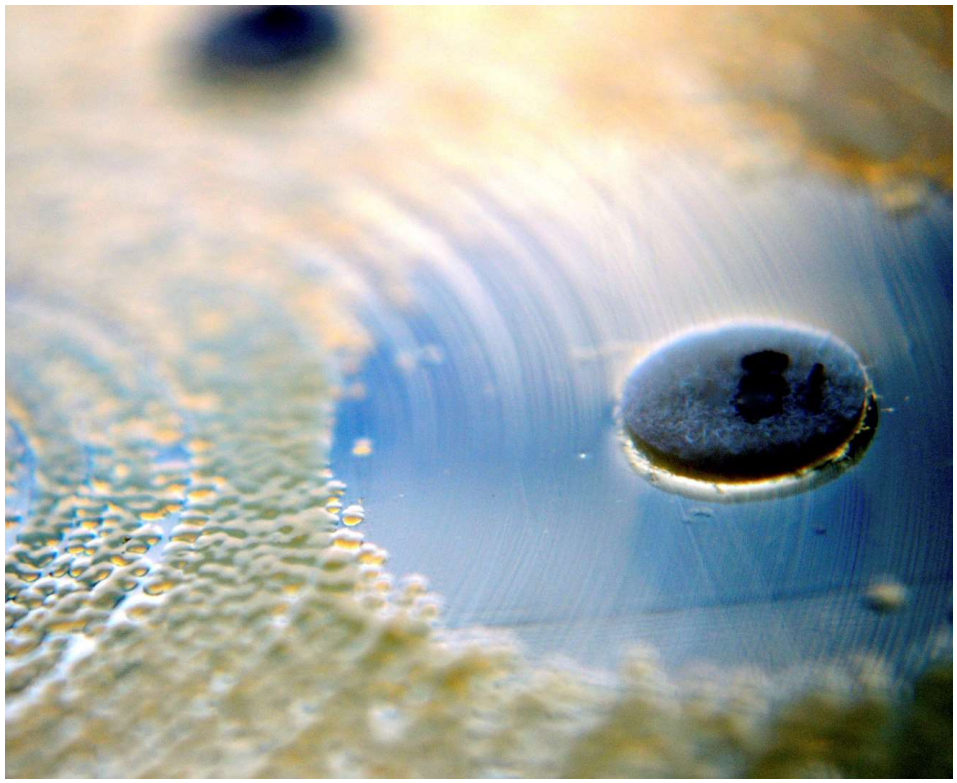
What Is Antimicrobial Resistance?

Antimicrobials: a drug or other agent used to treat infectious disease by inhibiting growth or killing the microorganism responsible for infection.

Antibacterials · Antivirals · Antifungals · Antiparasitics

Antimicrobial resistance (AMR): ability of a microorganism to avoid the effects of antimicrobials



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Antibiotics and Resistance Throughout History

Resistance has been with us all along

- Antimicrobial compounds are produced by bacteria, fungi, and plants
  - Streptomycin: a potent, broad-spectrum antibiotic produced by *Streptomyces* bacteria (1),(2)
- Co-evolution of resistance: offense and defense
  - Late Pleistocene permafrost: 30,000 year old gene sequences with homology to resistance genes for tetracyclines, glycopeptides, and β-lactam antibiotics (3)
- Human medicinal use is ancient
  - Artemisinin: antimalarial compound produced by *Artemisia annua* plants (sweet wormwood) used in China for thousands of years (4)
  - Tetracyclines: broad-spectrum antibiotics produced by a variety of *Actinomycetes* soil bacteria; evidence of use found in human skeletal remains in ancient Sudanese tribes almost 2,000 years old (5),(6)

(1) Clardy, J. Curr Biol. June 9; 19(11): R437–R441 (2009)
(2) Perry, J. Cld Spg Harb Perspect Med 6:a025197 (2016)
(3) D'Costa, V., et al. Nature 477, 457–461 (2011)
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(5) Kobayashi, T., J Mol Evol (2007) 65:228–235 (2006)
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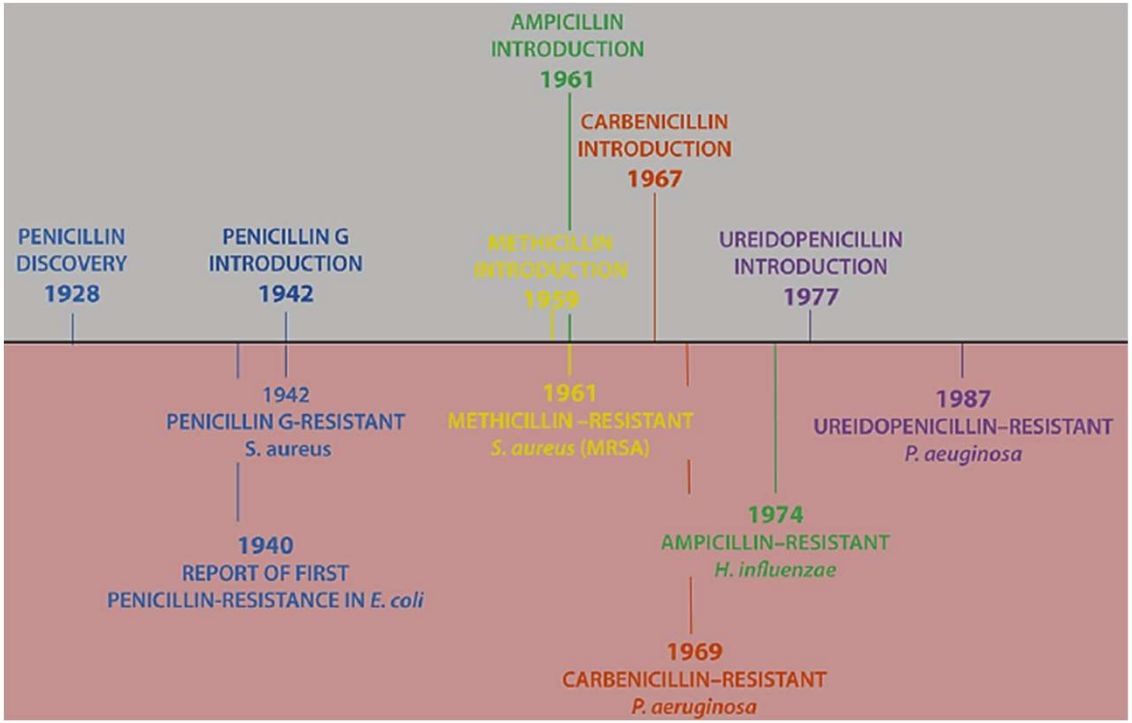
Modern Medicine: The Antibiotic Era

- Penicillin was first discovered in 1928 and developed for clinical use by 1940
- Penicillin was in wide use in many areas by 1940-1945, and reports of resistance in *Staphylococcus aureus* strains began in hospitals in 1942
- Methicillin, a 2nd-generation β-lactam compound, was introduced in 1961; resistance was reported by 1962
- *S. aureus* strains, previously resistant to penicillin, developed additional mechanisms for resistance and became MRSA



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The Rise of Resistance

Events of Note

Key Events:

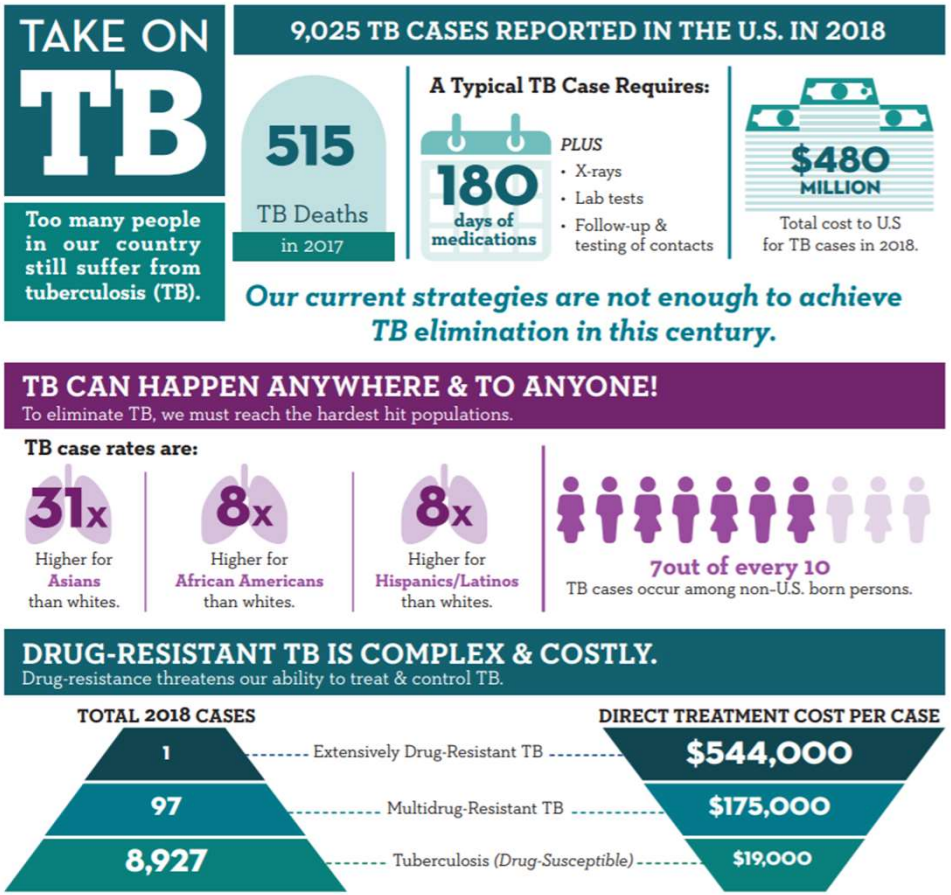
- 1962: Methicillin-resistant *Staphylococcus aureus* (MRSA)
- 1968: Erythromycin resistant *Streptococcus pneumoniae*
- 1988: Vancomycin-resistant *Enterococcus*
- 2000: Extensively-drug resistant Tuberculosis (XDR TB)
- 2002: Vancomycin-resistant *Staphylococcus aureus*
- 2004: Pan-drug resistant (PDR) *Acinetobacter* and *Pseudomonas*
- 2009: Pan-drug resistant (PDR) Enterobacteriaceae

www.amr.biomerieux.com

The Rise of Resistance

Key Examples – Mycobacterium tuberculosis

- 2nd most common cause of death due to infection after HIV/AIDS
- Estimated mortality in 2018: 1.5 million deaths globally
- Estimated cases globally: 1.8 billion people
- Cases of resistant infections in 2018: 0.48 million
- Likelihood of treatment success:
  - Drug-Susceptible TB: 83%
  - MDR-TB: 54% (resistant to at least two front-line antibiotics)
  - XDR-TB: 30% (resistant to at least two front-line and two second-line antibiotics)
  - XDR-TB has been isolated in more than 127 countries



www.cdc.gov

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CDC. 2019 AR Threats Report. CDC, HHS (2019)

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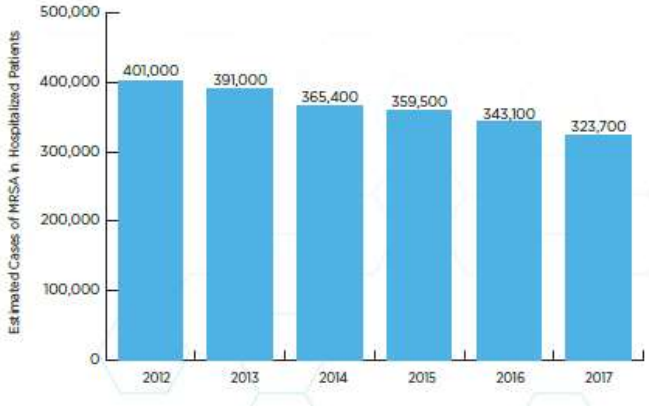
The Rise of Resistance

Key Examples – Methicillin-resistant Staphylococcus aureus (MRSA)

- Causes infections in the skin and soft-tissue (SSI), the endocardium, bloodstream, respiratory tract, bones, joints, and central nervous system
- Estimated cases do not include many SSIs
- MRSA infection rates were slowing, but progress has stalled

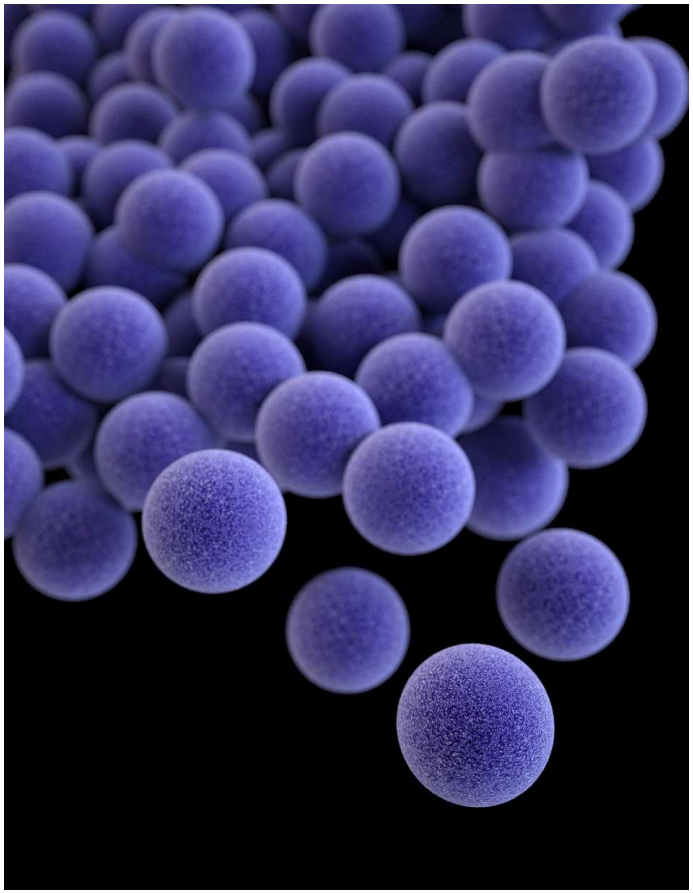
Cases represented do not include the many skin infections that happen, but are not cultured and diagnosed.



CDC. 2019 AR Threats Report. CDC, HHS (2019)

Present Day: The Post-Antibiotic Era

- Antimicrobials are necessary for infections and to ensure the safety of modern medical procedures
  - 1.7M adults develop sepsis every year in the US
  - 1.2M cesarean sections were performed in 2017
  - 30M people have diabetes and are at higher risk for infection
  - 33,000 organ transplants were performed in 2016
  - 500,000 people received dialysis treatment in 2016
  - 650,000 people receive cancer chemotherapy each year
- 'Superbugs' and the new era
  - Multidrug resistance: MDR pathogens resistant to multiple antimicrobials through acquired mechanisms
  - Pan-drug resistance: PDR pathogens resistant to all available antimicrobials through acquired mechanisms (untreatable)



CDC. 2019 AR Threats Report. CDC, HHS (2019)

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How Antibiotics Work

Classification by subcellular target

- Cell wall
- Cell membrane
- Protein biosynthesis
- Transcription
- Translation
- Other pathways

Target	Class	Sub-Class	Mechanism of Action	Compounds (Examples)
Cell Wall	β-lactams	Carbapenems Cephalosporins Monobactams Penicillins	Peptidoglycan biosynthesis inhibition	Ampicillin
Cell Wall	Other	Glycopeptides Novel Classes	Inhibition of one of several steps in peptidoglycan biosynthesis	Vancomycin
Cell Membrane	Cyclic Peptides	Polymyxins	Disrupts membrane permeability	Colistin
Protein Biosynthesis	30S	Aminoglycosides Tetracyclines	Inhibition of translation through 30S rRNA	Streptomycin
Protein Biosynthesis	50S	Macrolides Lincosamides Streptogramins Chloramphenicol	Inhibition of translation through 50S rRNA	Erythromycin
Protein Biosynthesis	Peptide Bond Formation	Oxazolidinones Mupirocin	Inhibition of translation through initiation or elongation disruption	Linezolid
Nucleic Acids	Transcription	Rifamycins	Inhibition of transcription through RNA polymerase disruption	Rifampin
Nucleic Acids	DNA Replication	Quinalones	Inhibition of the DNA replication fork through disruption of type II topoisomerases	Ciprofloxacin
Nucleic Acids	DNA Structure	Nitroimidazoles Nitrofurans	Disruption of DNA structure through the production of free radicals	Metronidazole
Other Pathways	THF Synthesis	Sulfonamides Trimethoprim	Inhibition of tetrahydrofolic acid (THF) synthesis	Sulfamethoxazole

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How Antibiotics Work: Cellular Integrity

- Cell walls: Gram-positive and Gram-negative bacteria
- Peptidoglycan synthesis
- Membrane permeability

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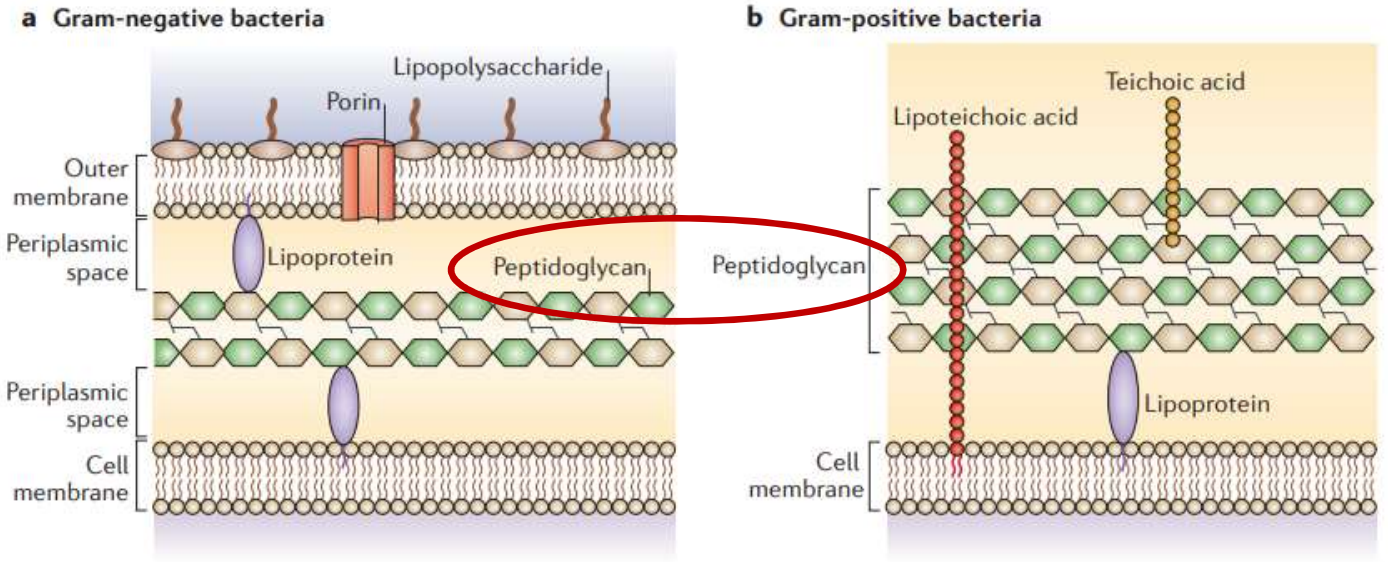

Brown, L., Nat Rev Microbiol. Oct;13(10):620-30 (2010)

How Antibiotics Work: Carbapenems

- Carbapenems: Activity of Imipenem in *Escherichia coli*
- Penicillin Binding Protein (PBPs) targets of Imipenem: PBP-1A, -1B, -2, -4, -5

Cell Wall	β-lactams	Carbapenems Cephalosporins Monobactams Penicillins	Peptidoglycan biosynthesis inhibition	Ampicillin
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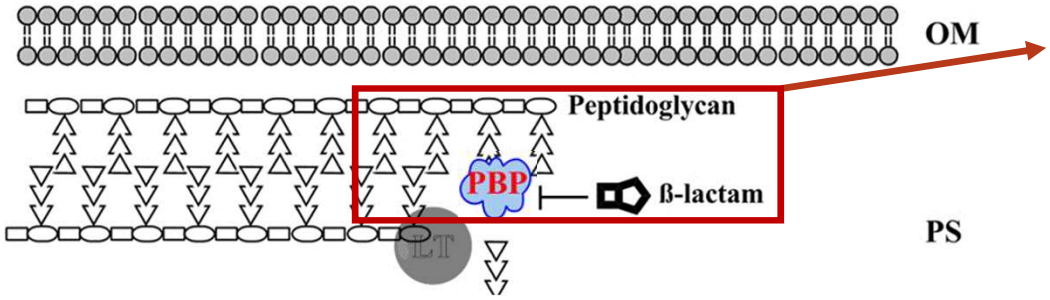

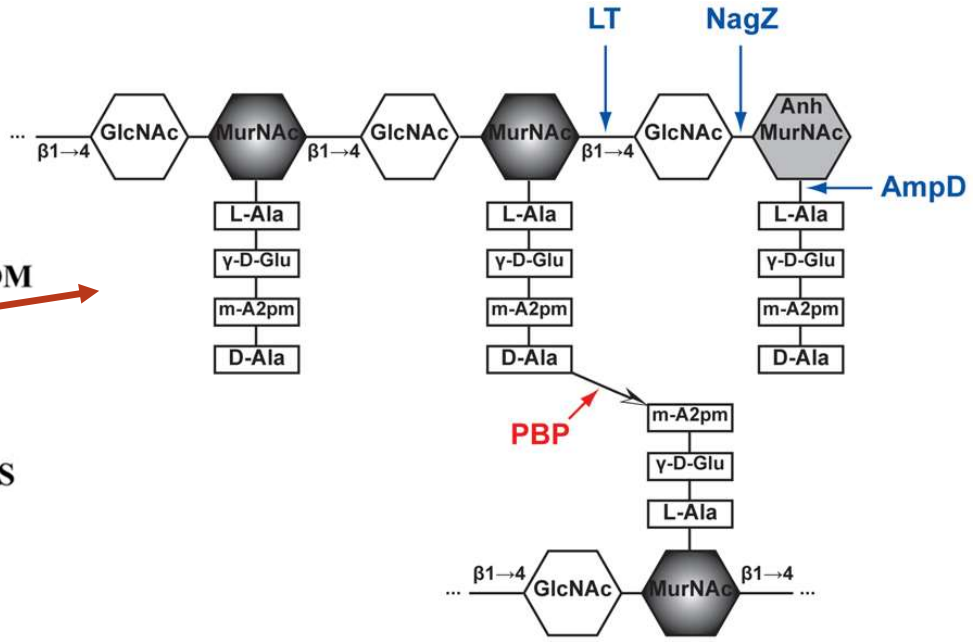

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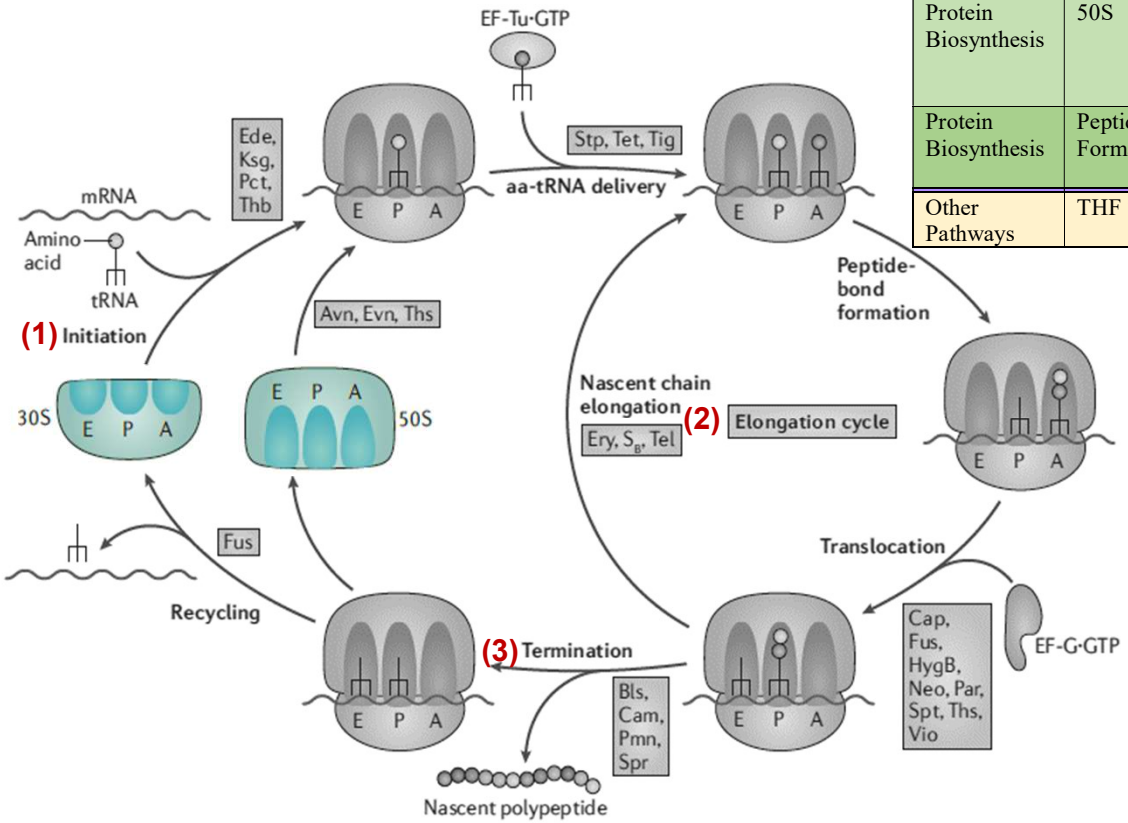
How Antibiotics Work: Protein Biosynthesis



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Other Pathways	THF Synthesis	Sulfonamides Trimethoprim	Inhibition of tetrahydrofolic acid (THF) synthesis	Sulfamethoxazole

- Protein biosynthesis: three steps of peptide translation
  - Initiation (1)
  - Elongation (2)
  - Termination (3)
- Ribosomal subunits
  - 30S
  - 50S

Wilson, D., Nat Rev Microbiol 12, 35–48 (2014)

How Antibiotics Work: Macrolides

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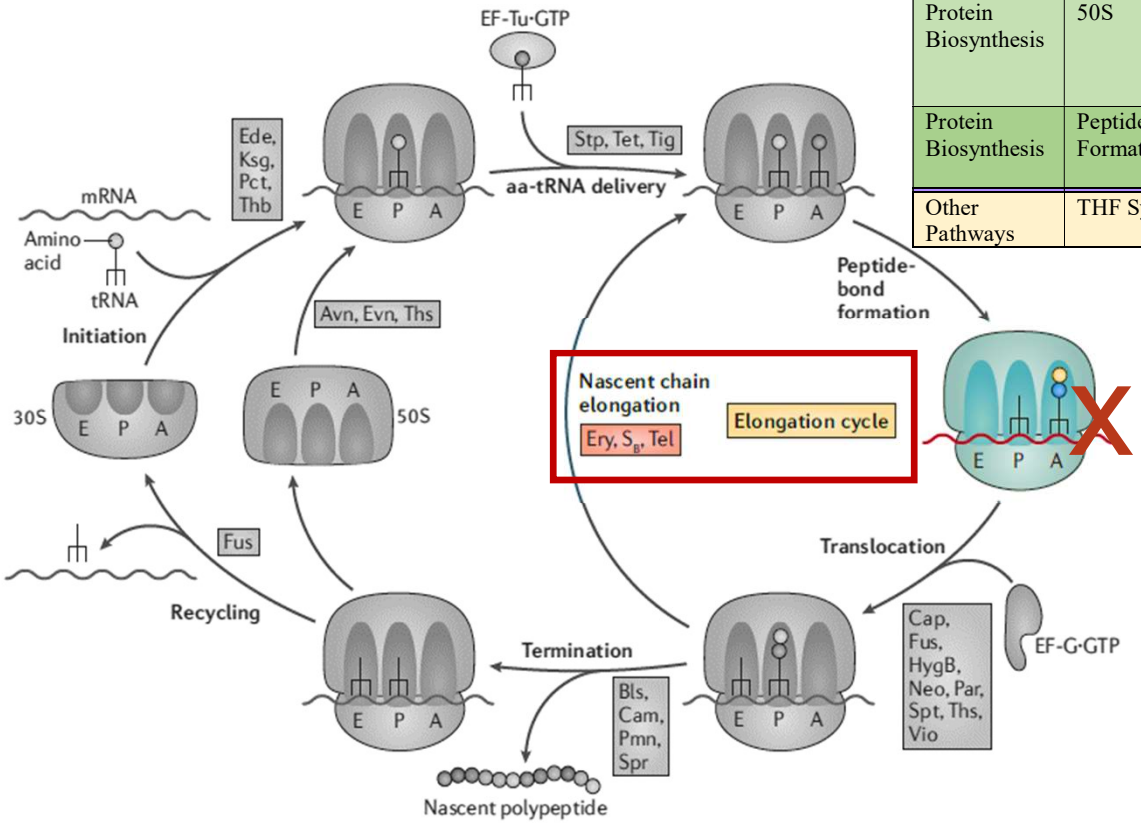

- Macrolides: Erythromycin in *Streptococcus pneumoniae*
- Erythromycin (Ery) binds the 50S subunit, causing premature translation termination

Wilson, D., Nat Rev Microbiol 12, 35–48 (2014)

How Antibiotics Work: Nucleic Acids

- DNA replication:
  - Chromosomal tension
- Topoisomerases
  - Type II: cleave both DNA strands

Nucleic Acids	Transcription	Rifamycins	Inhibition of transcription through RNA polymerase disruption	Rifampin
Nucleic Acids	DNA Replication	Quinalones	Inhibition of the DNA replication fork through disruption of type II topoisomerases	Ciprofloxacin
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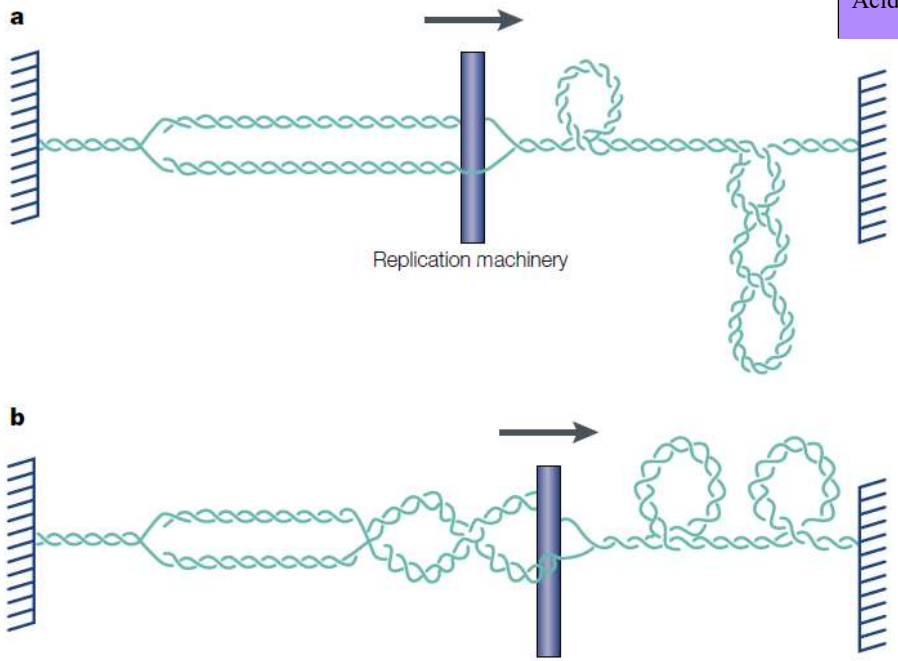

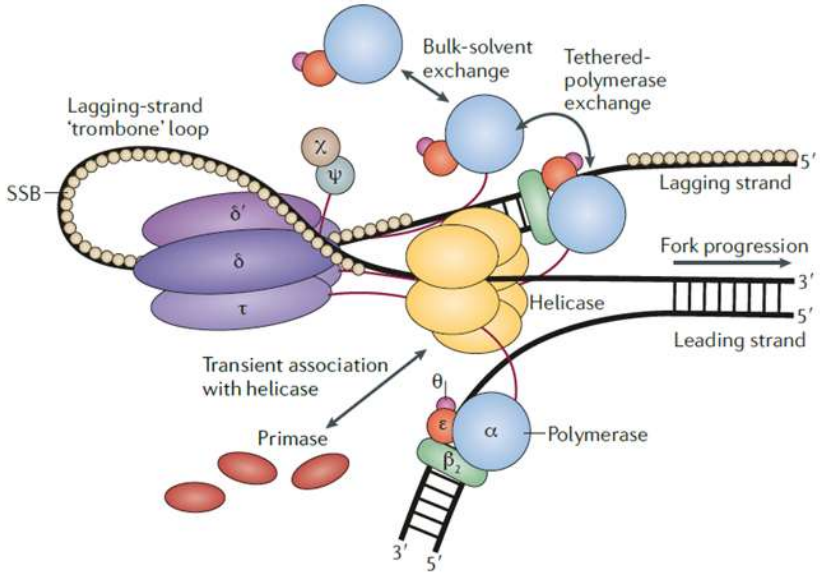

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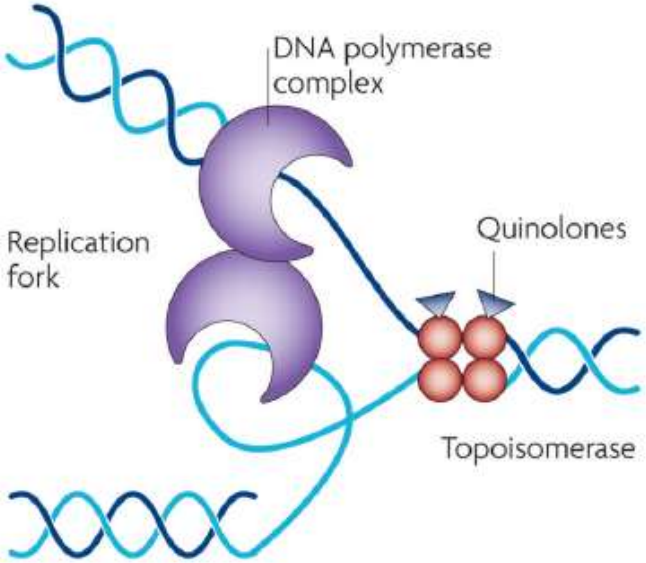
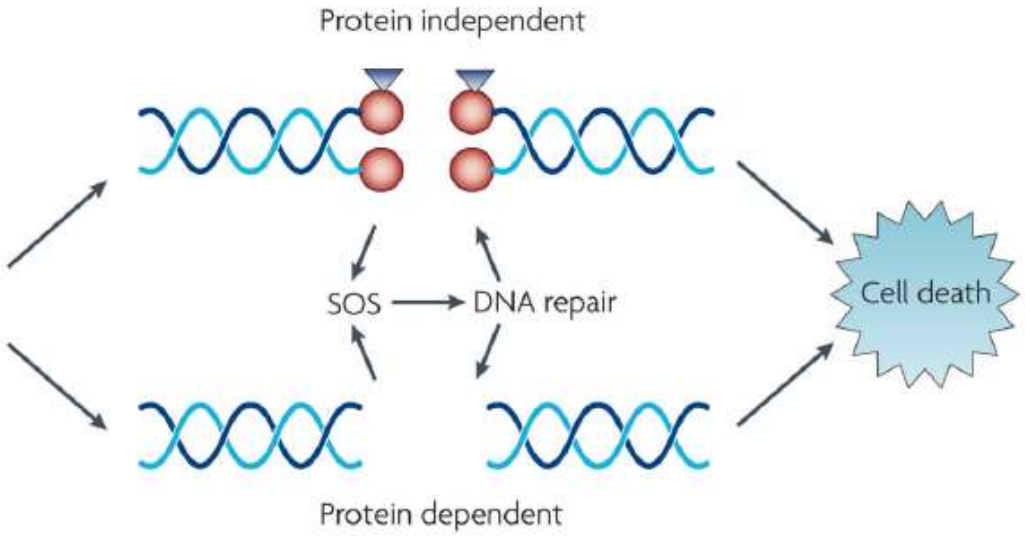
How Antibiotics Work: Quinolones

- Quinolones: Ciprofloxacin in *Escherichia coli*
- Ciprofloxacin binds DNA gyrase at the DNA replication fork

Nucleic Acids	Transcription	Rifamycins	Inhibition of transcription through RNA polymerase disruption	Rifampin
Nucleic Acids	DNA Replication	Quinalones	Inhibition of the DNA replication fork through disruption of type II topoisomerases	Ciprofloxacin
Nucleic Acids	DNA Structure	Nitroimidazoles Nitrofurans	Disruption of DNA structure through the production of free radicals	Metronidazole



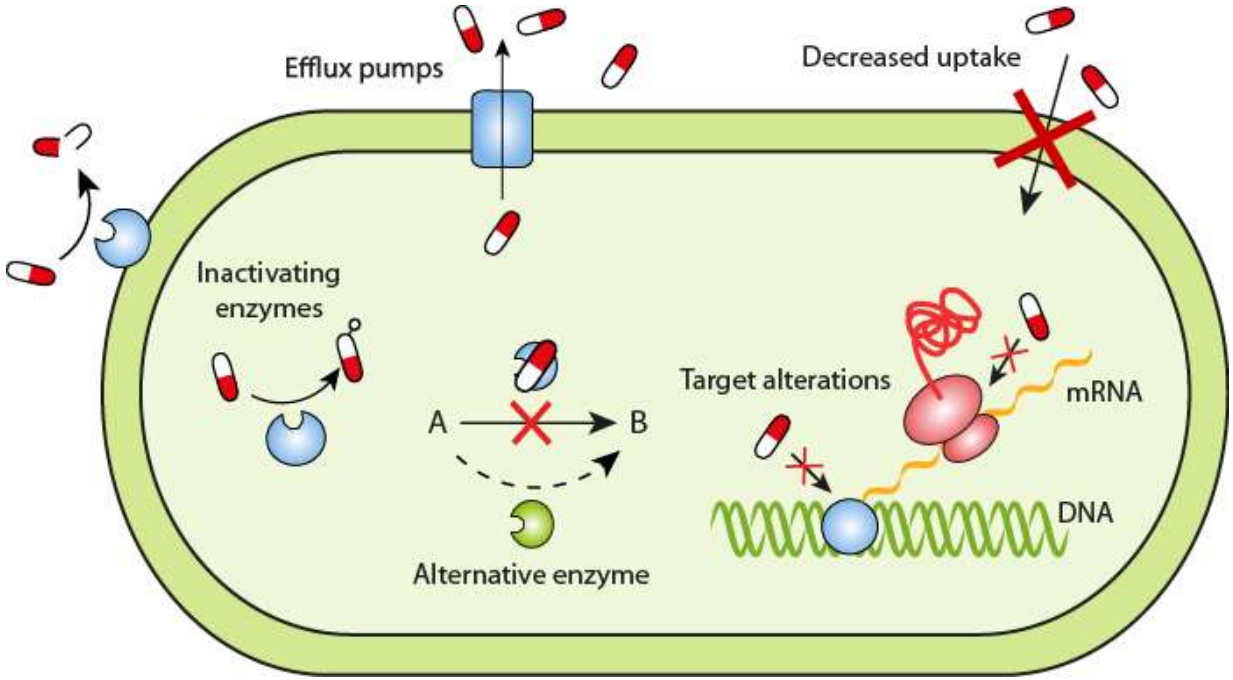


Kohanksi, M., Nat Rev Microbiol. June 8(6): 423–435 (2018)

Types of Resistance Mechanisms

Types of resistance mechanisms:

- Decreased influx, accumulation, or uptake
- Active efflux pumps
- Enzymatic inactivation or destruction
- Modification to the antimicrobial target site



DOI: 10.13140/RG.2.2.27592.72967

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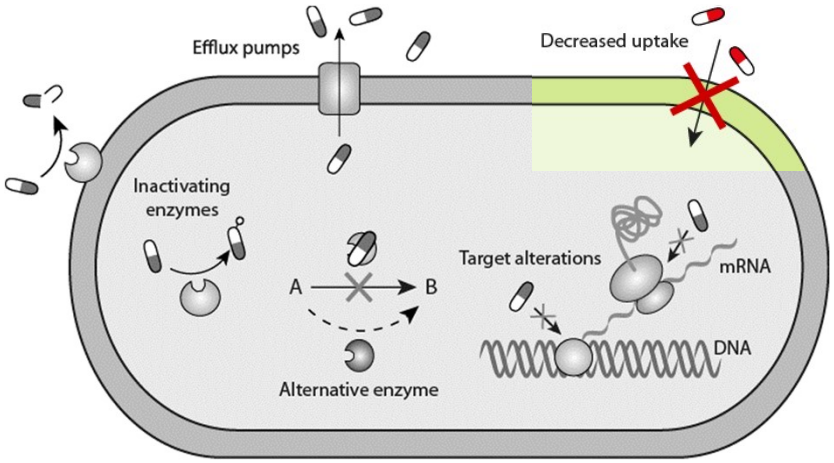
How Resistance Works: Decreased Influx

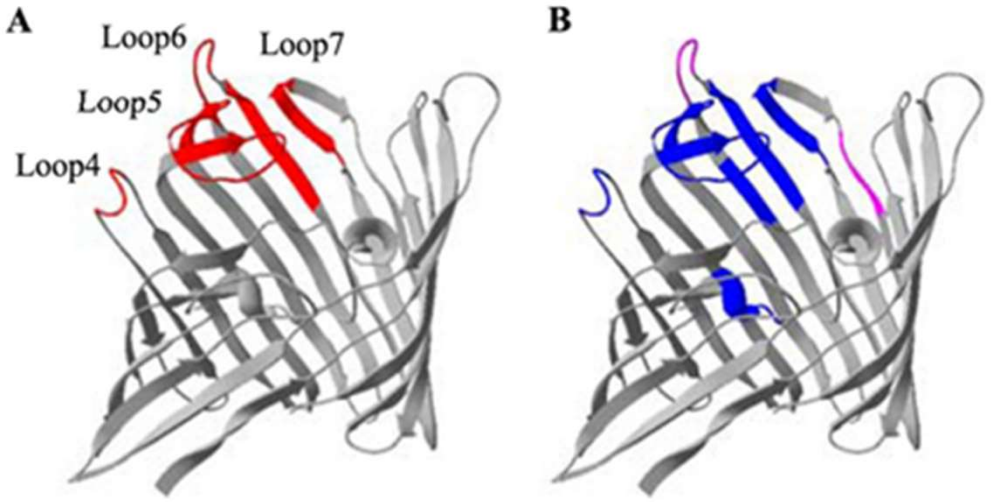
Porins: mediators of permeability

- Porin loss: transcription control, sequence mutation
- Porin function: channel conformation, size



Porin-mediated resistance in *Neisseria gonorrhoeae*

- PIB porin: mutations in *penB* gene change porin conformation
- Confers resistance to tetracyclines, penicillins, fluoroquinalones



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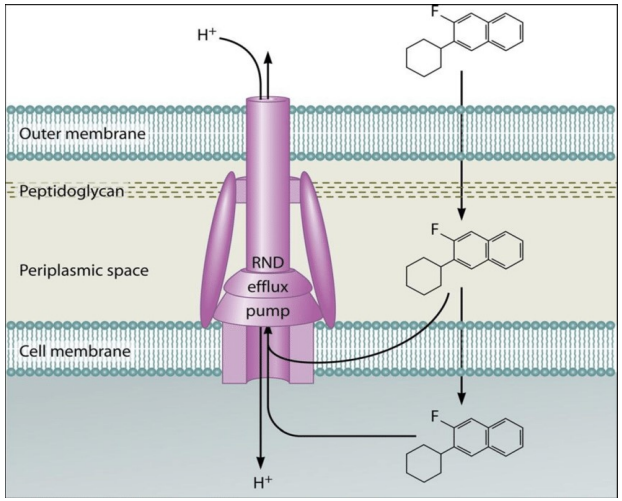
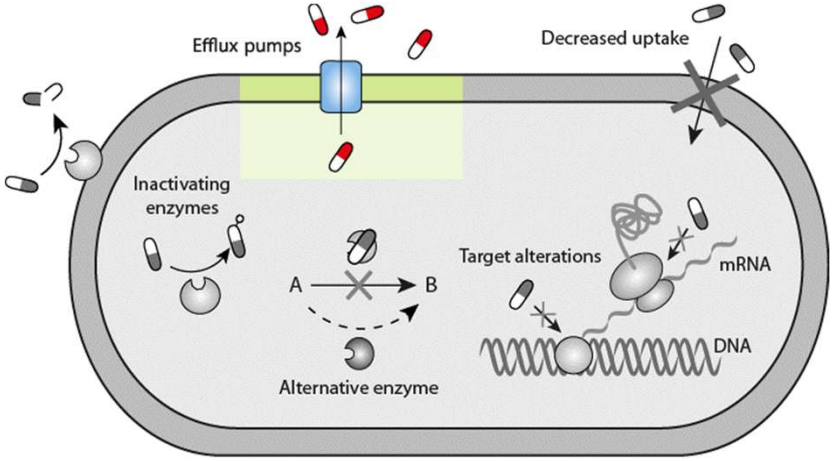
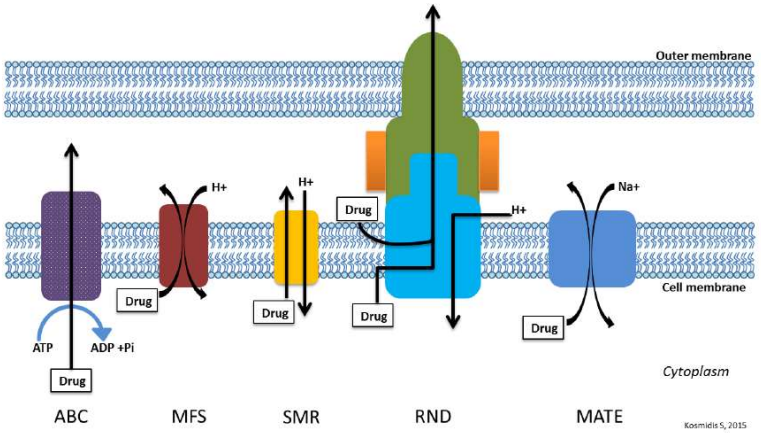
How Resistance Works : Active Efflux

Efflux pump-mediated resistance in *Pseudomonas aeruginosa*

- MexAB-OprM system: RND (Resistance-nodulation-division) family of efflux pumps
- Multidrug efflux operon with homologs in many other pathogens
- Confers resistance to fluoroquinalones, macrolides, chloramphenicol, tetracyclines, β-lactams





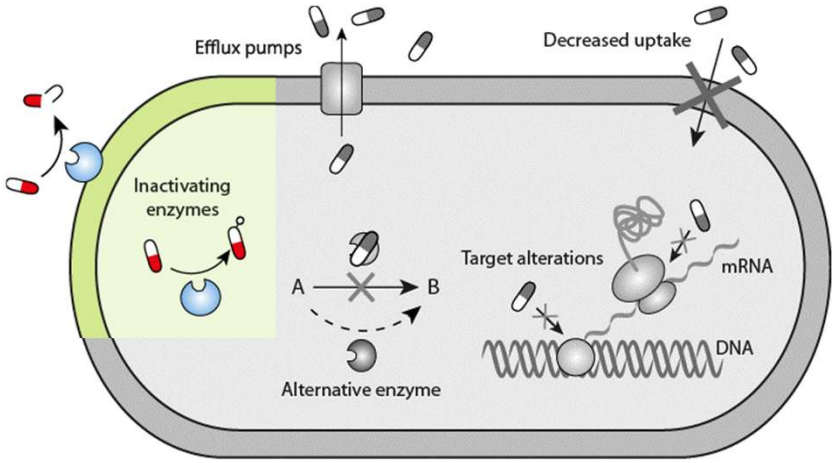


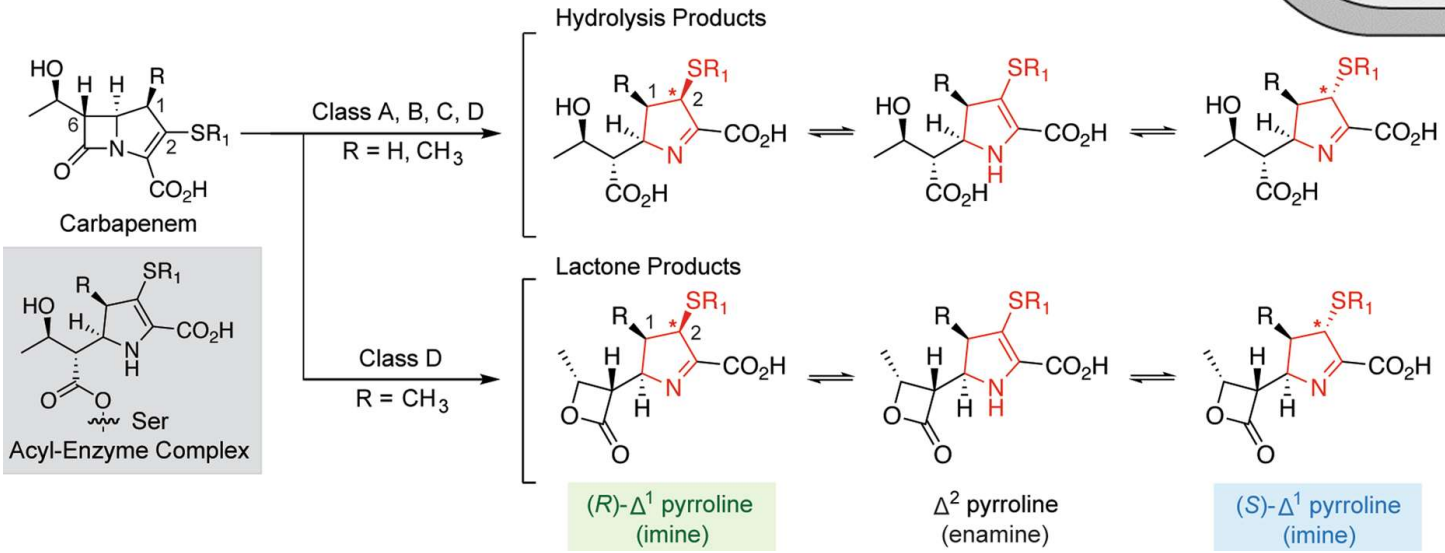
Reygaert, W., AIMS Microbiology, 4(3): 482–501 (2018)

How Resistance Works : Enzymatic Inactivation

Extended-spectrum β-lactamase (ESBL) enzyme-mediated resistance in *Enterobacteriaceae*

- ESBL type CTX is a class A β-lactamase found in *Escherichia coli*, *Salmonella enterica*, and other species
- Confers resistance to β-lactams; often cross-resistant to quinolones and trimethoprim/sulfamethoxazole



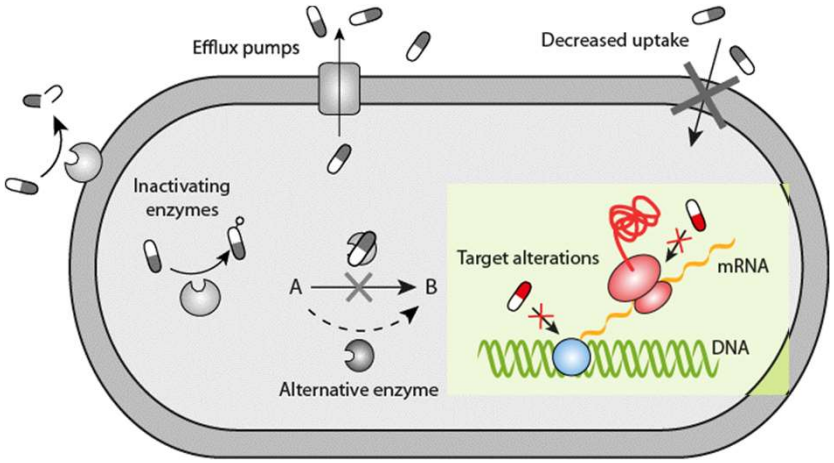


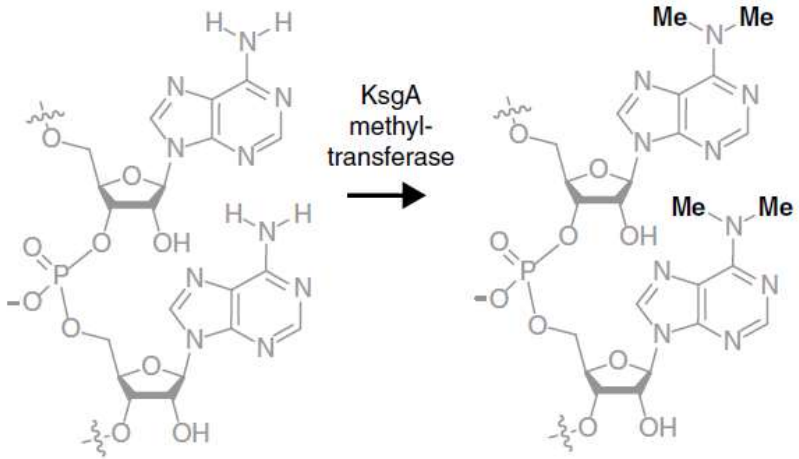
Lohans, C.T., Sci Rep 9, 13608 (2019)

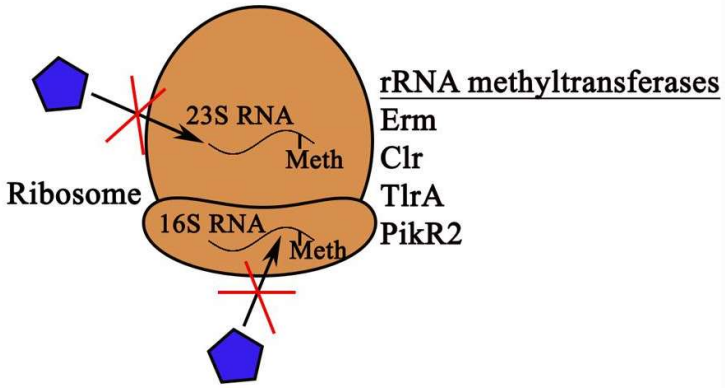
How Resistance Works: Target Site Modification

Methylated rRNA-mediated resistance in *Streptococcus pneumoniae*

- Methyltransferases: *erm* gene products catalyzes methylation of 50S subunit at different sites, changing macrolide binding site recognition
- Confers resistance to macrolides, lincosamides, and streptogramin (MLS antimicrobials)







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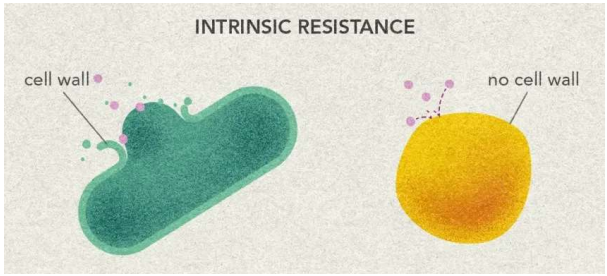
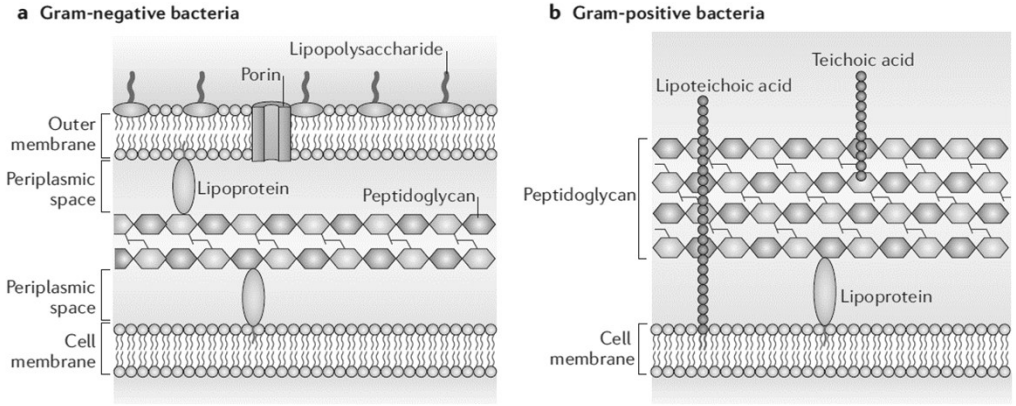
Ranasinghe, R.T., Nat Commun 9, 655 (2018)

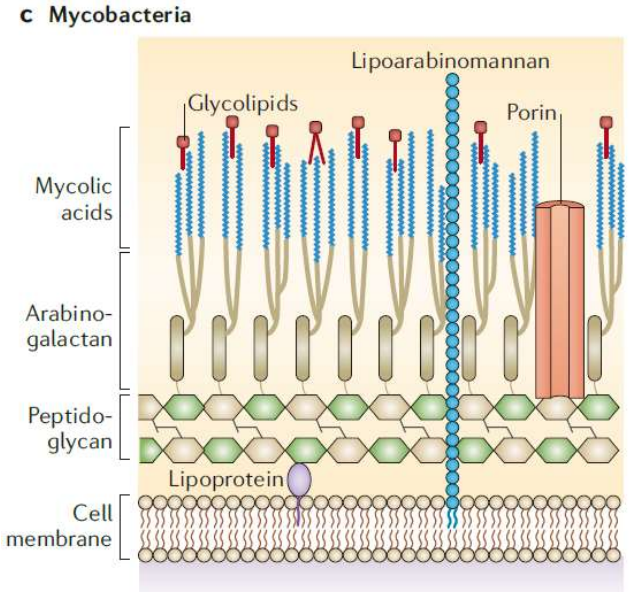
Spread of Resistance: Intrinsic Resistance

Intrinsic resistance is the innate ability of an organism to resist the action of an antimicrobial as a result of structural or functional characteristics

- Lack of susceptible physiology
- Permeability barriers







www.microbeonline.com
Brown, L., Nat Rev Microbiol. Oct;13(10):620-30 (2010)

Spread of Resistance: Acquired Resistance

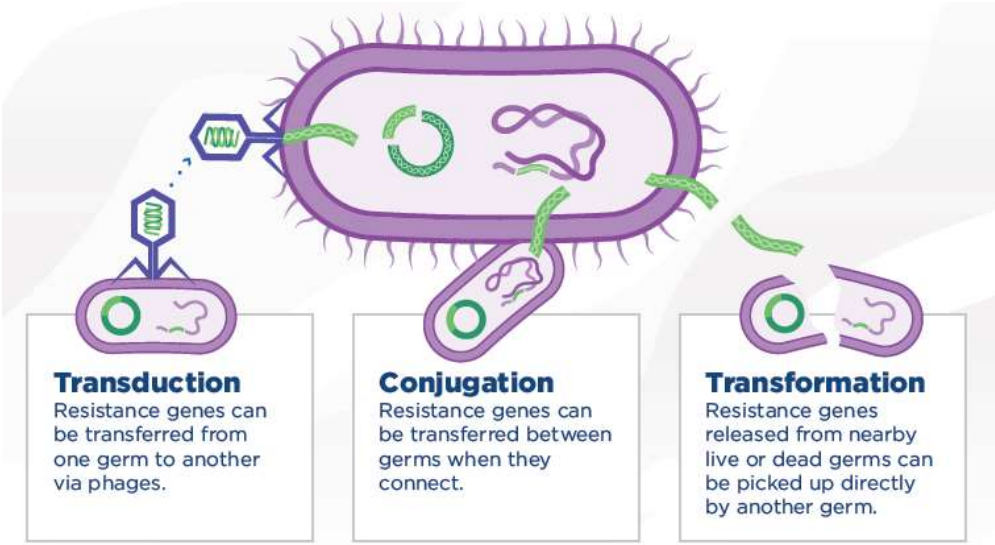
Acquired Resistance – Horizontal Gene Transfer

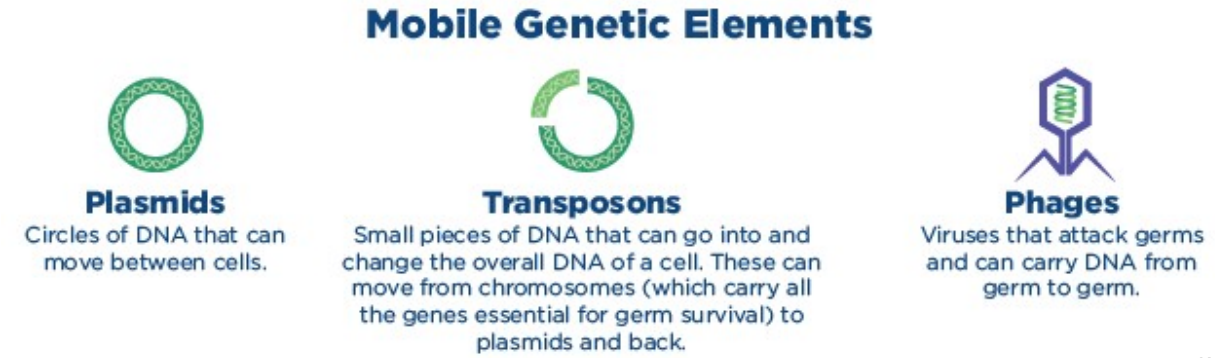
Horizontal gene transfer (HGT)

- Transformation: DNA
- Transduction: Phage
- Conjugation: Pilus

Mobile genetic elements (MGE)

- Plasmids
- Transposons
- Mobile gene cassettes
- Phage DNA





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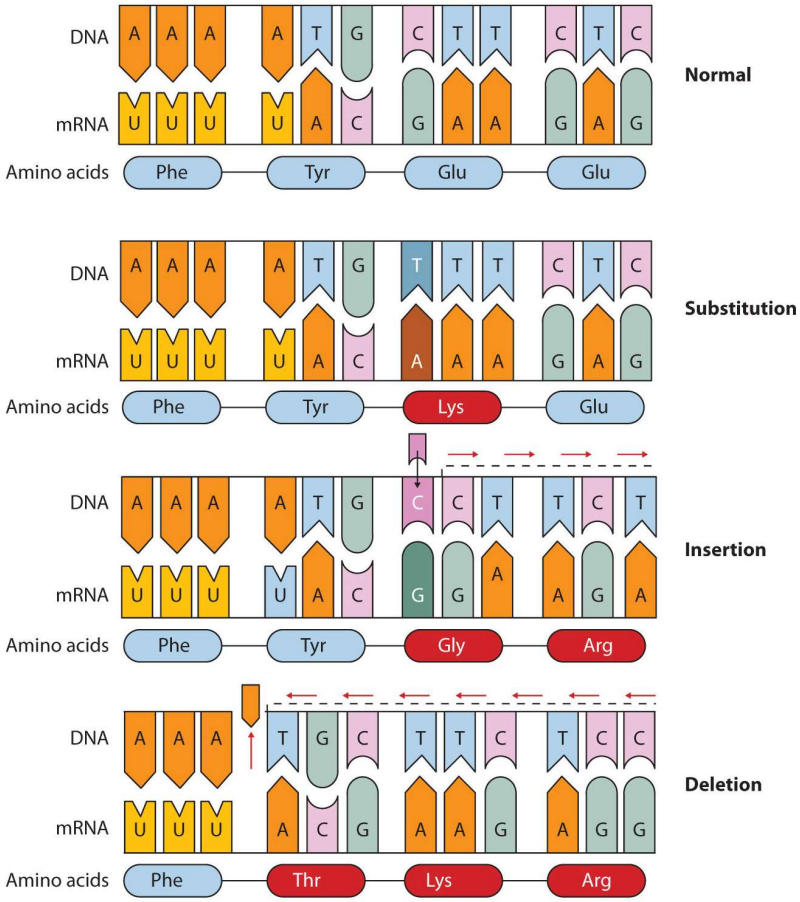
Genetics of Resistance

Acquired Resistance - Mutation

- Mutation rates in prokaryotes: approximately 10^{-6}/base, per generation
- Point mutations – single base replacements
- Deletion – the removal of nucleotide bases or sequences
- Duplication – the production of one or more copies of a genetic sequence
- Inversion – the reversal of a genetic sequence
- Insertion – the addition of bases or sequences
- Translocation – the rearrangement of a genetic sequence



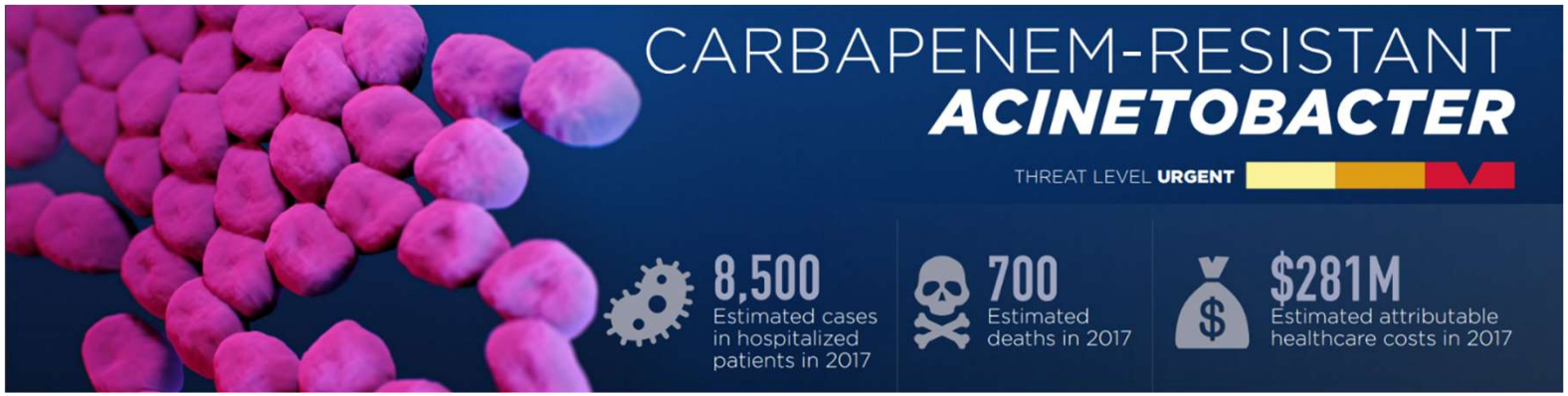
Urgent Threats

Carbapenem-resistant Acinetobacter baumannii

- *Acinetobacter* infections typically occur in health care settings and can be transmitted from person-to-person or contact with contaminated surfaces
- Pathology: infections in the blood, urinary tract, lungs (pneumonia), or wounds
- Several known mechanisms of resistance, including:
  - β-lactamases
  - Efflux pumps
  - Permeability defects
  - Aminoglycoside-modifying enzymes
  - Alteration of target sites
  - Inducible DNA damage response

PERCENT OF GERMS THAT TESTED NON-SUSCEPTIBLE (NOT SENSITIVE) TO OTHER TYPES OF ANTIBIOTICS

Select Antibiotics	2013	2014	2015	2016	2017
Any fluoroquinolone	98%	93%	97%	92%	89%
Any extended-spectrum β-lactam	80%	75%	81%	79%	75%
Ampicillin/sulbactam	62%	62%	59%	64%	61%
Trimethoprim/ sulfamethoxazole	84%	74%	81%	77%	66%



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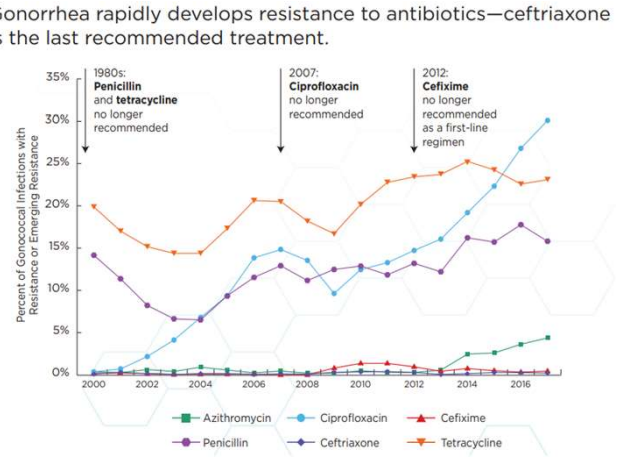
Urgent Threats

Multidrug-resistant Neisseria gonorrhoeae

- Gonorrhea has developed resistance to all but one class of antibiotics
- Pathology: a sexually transmitted infection (STI) that causes infertility, ectopic pregnancy, increased risk of HIV, and cardiovascular and nervous system complications
- Several known mechanisms of resistance, including:
  - β-lactamases
  - Chromosomal mutations
  - Efflux pumps
  - Changes in cell membrane permeability
  - rRNA methylases



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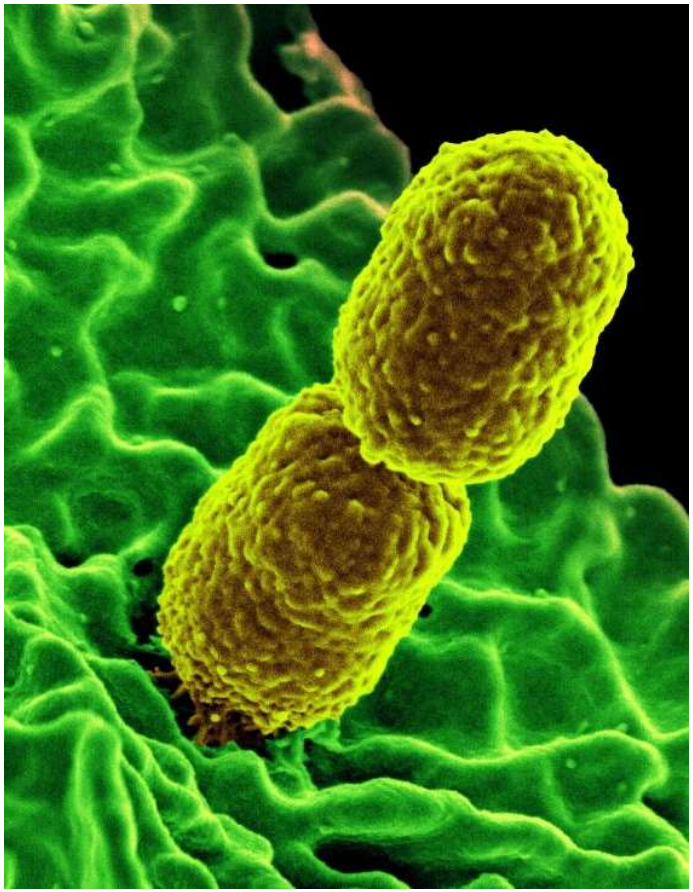
Summary

- Antimicrobial resistance is a growing global health concern that threatens our ability to treat infection and perform essential medical procedures
- There are numerous mechanisms of resistance that have emerged throughout the years
- Resistance can occur due to the innate ability to resist the action of antimicrobials, or through acquired resistance via HGT or mutation
- With numerous multidrug-resistant strains emerging, it is more important than ever that new therapeutics and novel detection methods are developed



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Cultivating collaboration to support global health

Upcoming Webinar:

Antimicrobial Resistance: Arm Your Lab in the Fight Against Superbugs

- Presented by Christine Fedorchuk, Ph.D.
- February 27, 12:00 ET

Learn more:

www.atcc.org/globalprioritysuperbugs

www.atcc.org/superbugs