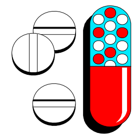
Antibiotic Classification and Modes of Action



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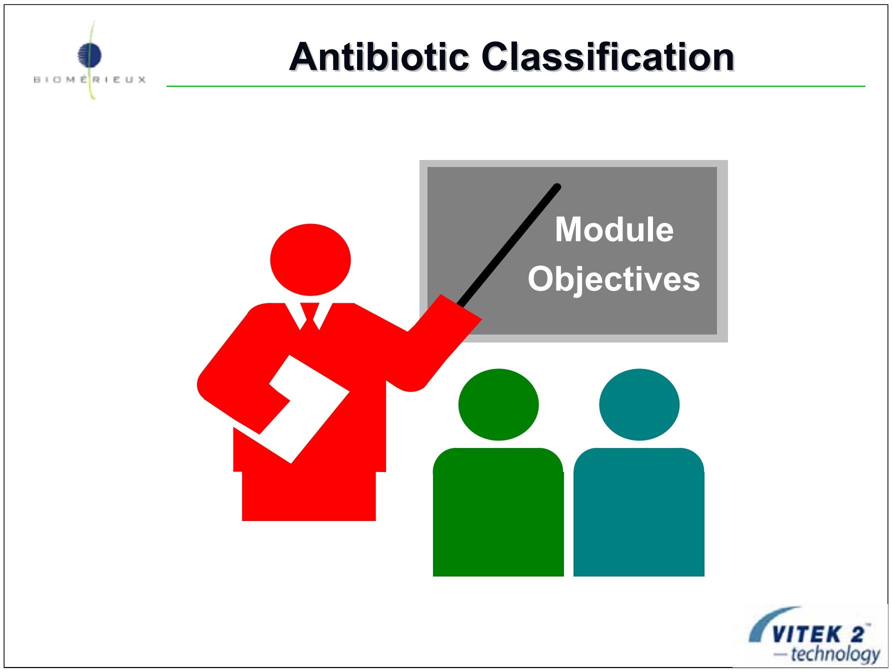
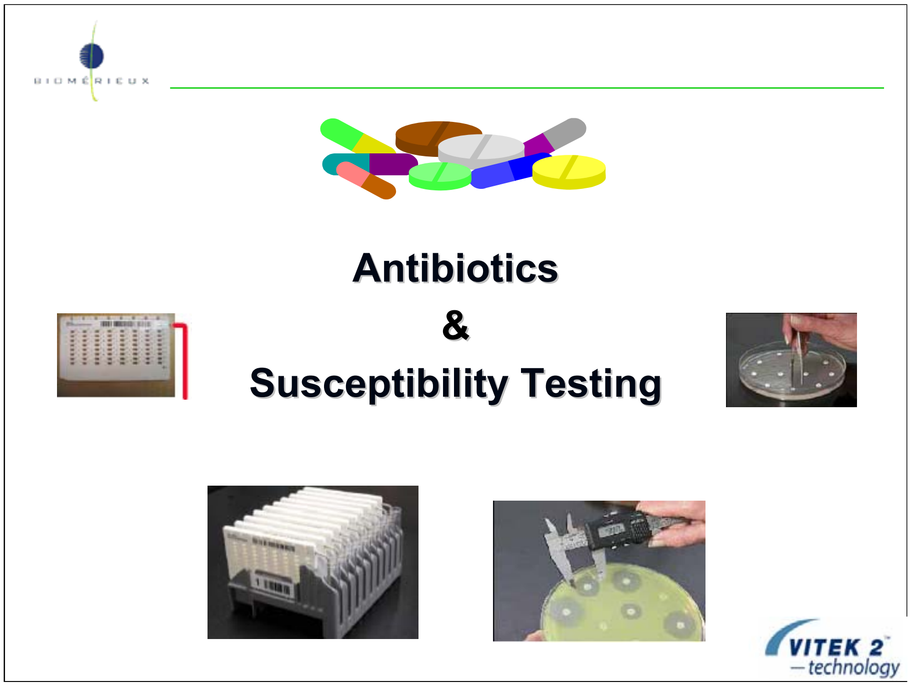


Upon completion of this module you will be able to:

- Explain why susceptibility testing is done
- Define the terms, bacteriostatic and bactericidal
- Describe the functional antibiotic classification scheme and list the 5 main groups
- Name at least one antibiotic in each class
- Describe the structure of a Gram-positive and negative cell
- Explain the modes of action for the antibiotics in each of the five functional antibiotic classes
- List examples of natural resistance in each of the five functional antibiotic classes
- Explain why it is not necessary to perform susceptibility testing for certain organism / antibiotic combinations

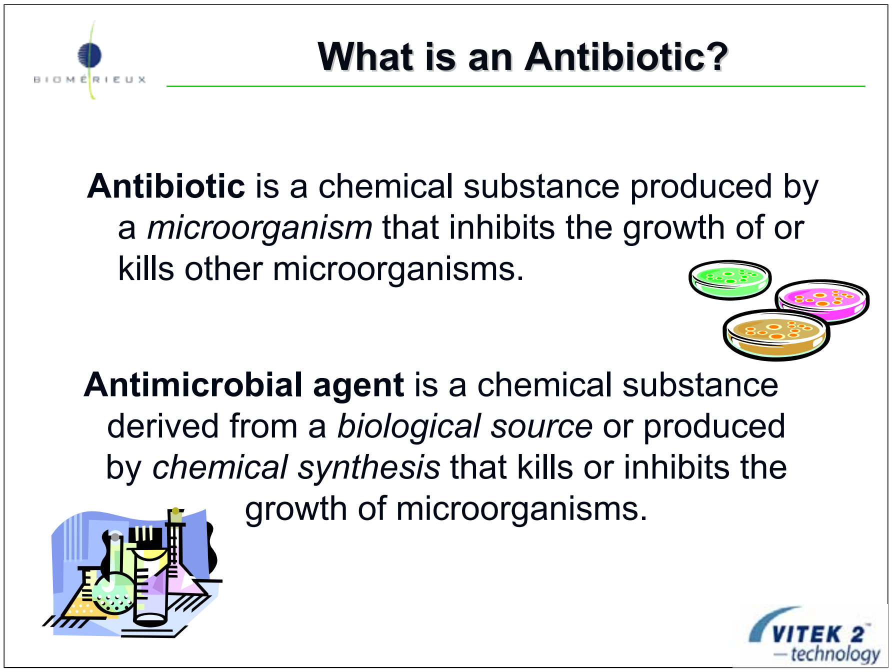


Microbiologists work with antibiotics every day.

Antimicrobial Susceptibility Testing (AST) is one of the primary functions of the Microbiology Lab.

But, how much do Microbiologists really know about antibiotics?

Let’s review some basic information and see how it can be applied daily.



The noun “antibiotic” was first used in 1942 by Dr. Selman A. Waksman, soil microbiologist. Dr. Waksman and his colleagues discovered several actinomycetes derived antibiotics.

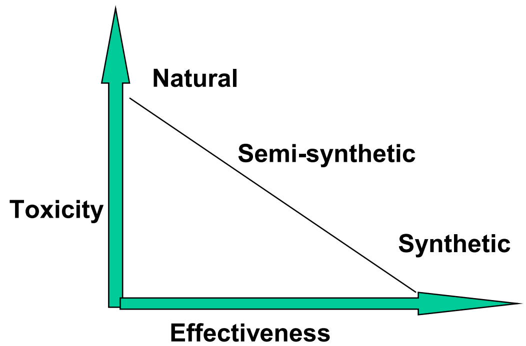
The two terms are usually used synonymously and that practice will continue throughout this presentation.

The word “antibiotic” will be used to describe:

> a chemical substance derivable from a microorganism or produced by chemical synthesis that kills or inhibits microorganisms and cures infections.

Sources of Antibacterial Agents

- **Natural** - mainly fungal sources
- **Semi-synthetic** - chemically-altered natural compound
- **Synthetic** - chemically designed in the lab



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- The original antibiotics were derived from fungal sources. These can be referred to as "natural" antibiotics
  - Organisms develop resistance faster to the natural antimicrobials because they have been pre-exposed to these compounds in nature. Natural antibiotics are often more toxic than synthetic antibiotics.
  - Benzylpenicillin and Gentamicin are natural antibiotics
- Semi-synthetic drugs were developed to decrease toxicity and increase effectiveness
  - Ampicillin and Amikacin are semi-synthetic antibiotics
- Synthetic drugs have an advantage that the bacteria are not exposed to the compounds until they are released. They are also designed to have even greater effectiveness and less toxicity.
  - Moxifloxacin and Norfloxacin are synthetic antibiotics
- There is an inverse relationship between toxicity and effectiveness as you move from natural to synthetic antibiotics

Role of Antibiotics

What is the role of antibiotics?

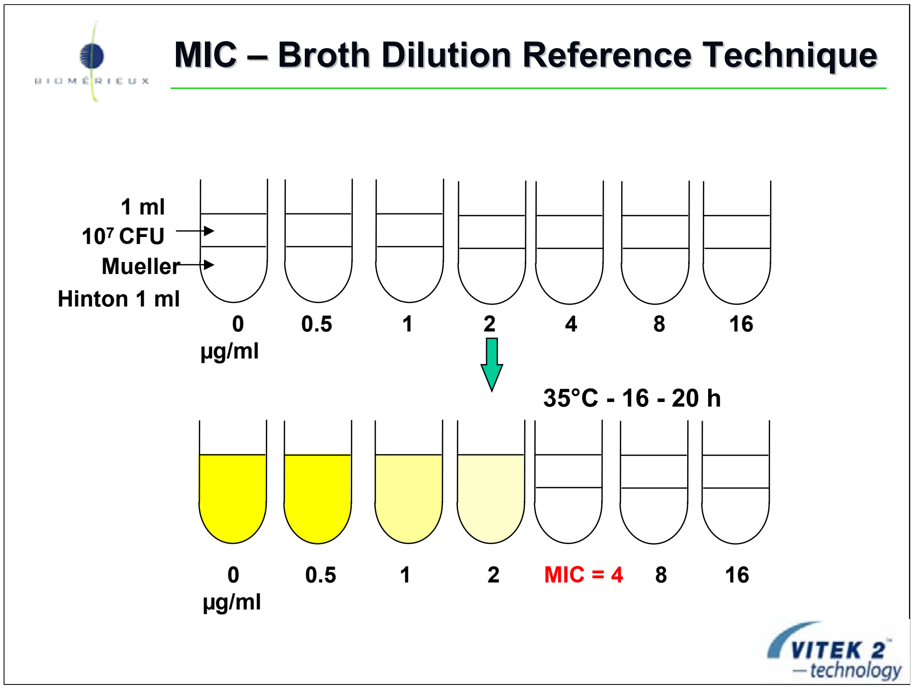
- To inhibit multiplication

Antibiotics have a bacteriostatic effect.

At which drug concentration is the bacterial population inhibited?

- Minimal Inhibitory Concentration = MIC

Bacteriostatic = inhibits bacterial growth



Quantitative Measure

- MIC = lowest concentration of antibiotic that inhibits growth (measured visually)
- Interpretation of quantitative susceptibility tests is based on:
  - relationship of the MIC to the achievable concentration of antibiotic in body fluids with the dosage given
  - For treatment purposes, the dosage of antibiotic given should yield a peak body fluid concentration 3-5 times higher than the MIC

    or

  - MIC x 4 = dosage to obtain peak achievable concentration

Role of Antibiotics

What is the role of antibiotics?

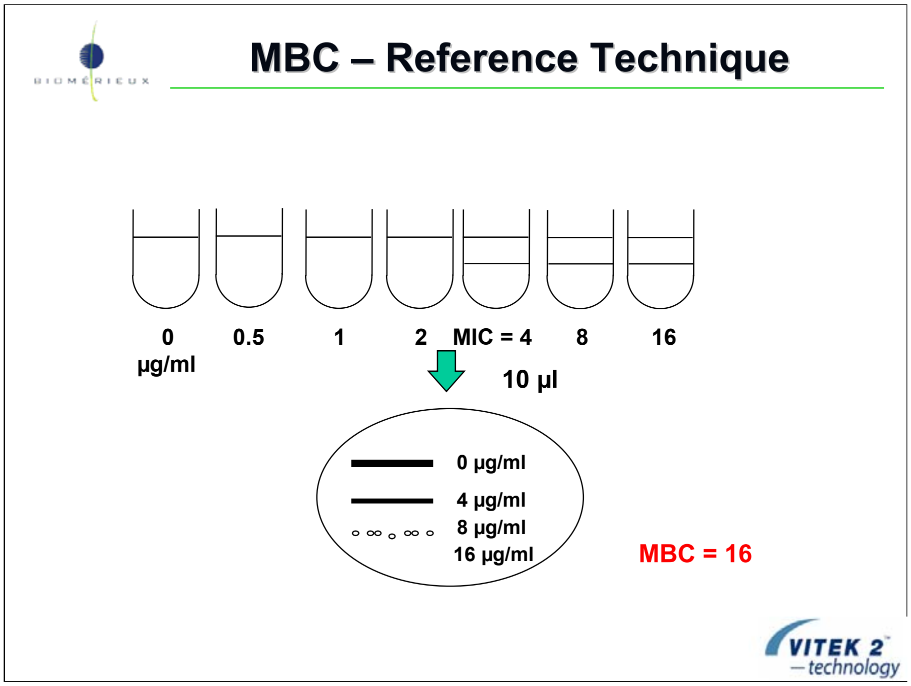
- To destroy the bacterial population

Antibiotics have a bactericidal effect.

At which drug concentration is the bacterial population killed?

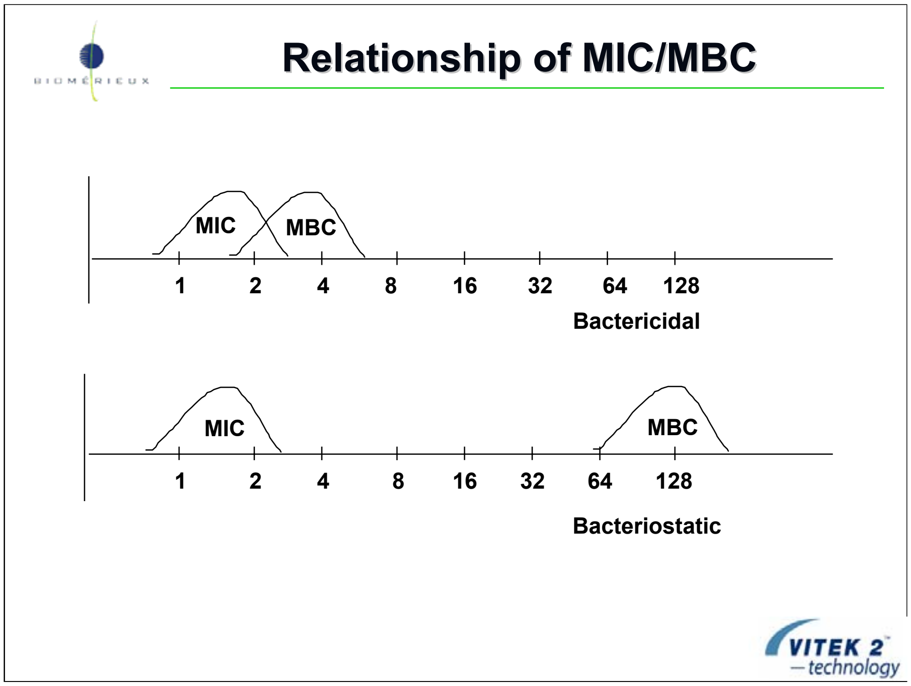
- Minimal Bactericidal Concentration = MBC

Bactericidal = kills bacteria



Quantitative Measure

- MBC = lowest concentration of antibiotic that kills bacteria



There is a much closer relationship between the MIC and MBC values for bactericidal drugs than for bacteriostatic drugs.

How Do Antibiotics Work?

Mechanisms of Action

Antibiotics operate by inhibiting crucial life sustaining processes in the organism:
the synthesis of cell wall material
the synthesis of DNA, RNA, ribosomes and proteins.

Target

The target of the antibiotic should be selective to minimize toxicity…but all antibiotics are toxic to some degree!

The targets of antibiotics should be selective to minimize toxicity.

- Selective Toxicity
    - Harm the bacteria, not the host

Why Do Susceptibility Testing?

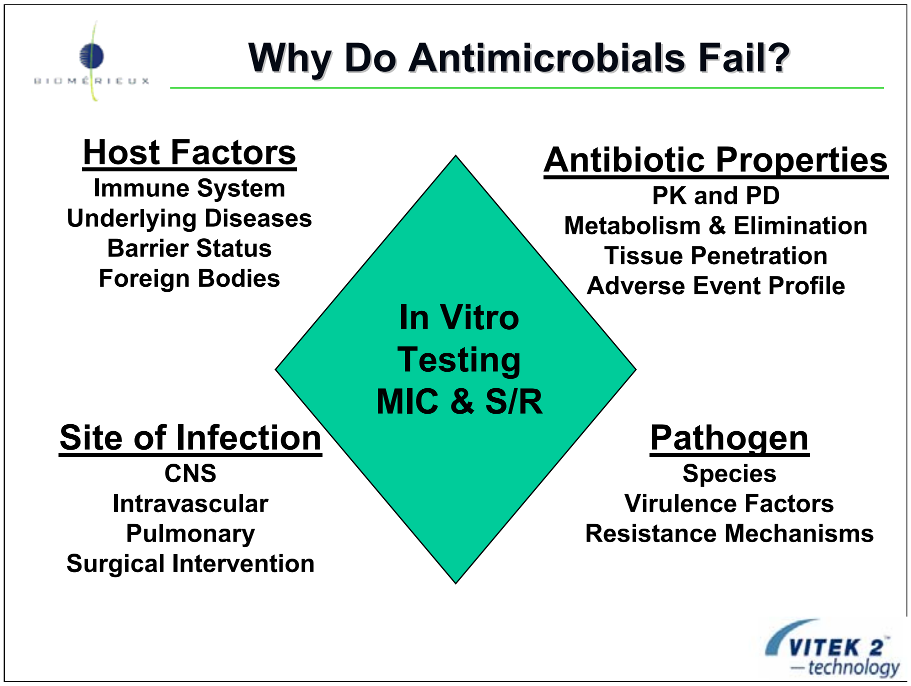
- Help patients today
  - To provide guidance to the physician in selection of antimicrobial therapy with the expectation of optimizing outcome
- Help patients tomorrow
  - Build an antibiogram to guide physicians in selecting empiric therapy for future patients with the expectation of optimizing outcome
- Help patients next decade
  - Drive new drug research
  - Monitor the evolution of resistance

We do this testing:

- To provide a guide for therapy
- Allows selection of the most appropriate agent
  - Least expensive
  - Narrowest spectrum
  - Most effective
- To monitor the evolution of resistance

Antibiotic resistance has an impact on individual health and public health.

- Types of resistance seen and frequency
- Which drugs you can expect to be successful
- Emerging and new resistance seen in the community



There are many possible reasons antimicrobials may fail.

Selection of the appropriate antibiotic depends on:

- knowledge of organism's natural resistance
- pharmacological properties of the antibiotic toxicity, binding, distribution, absorption achievable levels in blood, urine
- previous experience with same species
- nature of patients underlying pathology
- patient's immune status

Susceptibility testing focuses primarily on the interaction of antimicrobial agents, the organisms and their resistance mechanisms.

Interpreting Susceptibility Results

- MICs are not physical measurements
- There are many factors that play a role in determining clinical outcome
- Susceptibility in vitro does not uniformly predict clinical success in vivo
- Resistance will often, but not always, correlate with treatment failure

Susceptibility tests are essentially **artificial measurements.**

- in vitro response
- approximate range of effective inhibitory action
- possible error equivalent to one tube dilution

The only true measure of bacterial response to an antibiotic is the clinical response of the patient.

- outcome or in vivo response

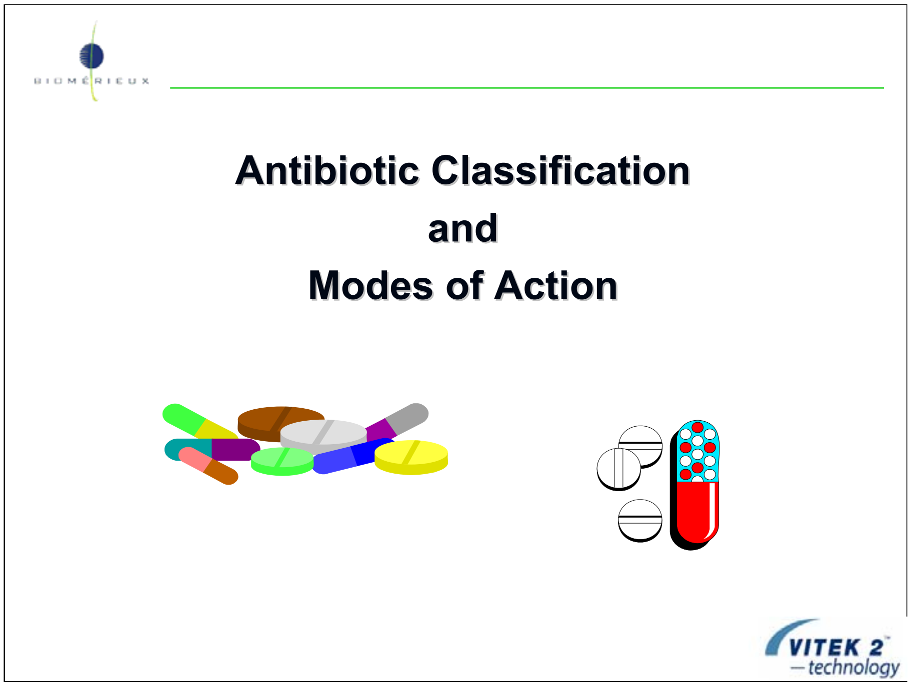
One of the real values of AST is to predict resistance

S = success likely, but no guarantee
R = correlates well with treatment failure

Is Resistance Testing a better name for what we do than Susceptibility Testing?

What is the Ideal Antibacterial?

- Selective target – target unique
- Bactericidal – kills
- Narrow spectrum – does not kill normal flora
- High therapeutic index – ratio of toxic level to therapeutic level
- Few adverse reactions – toxicity, allergy
- Various routes of administration – IV, IM, oral
- Good absorption
- Good distribution to site of infection
- Emergence of resistance is slow



In the AES Knowledge Base, phenotypes are organized by drug class.

The AES decision process attempts to identify a phenotype for each drug class tested. In order to understand and use the software effectively, it is important to have a solid working knowledge of antibiotic classification.

In addition, each drug class typically has a unique mode of action.

Bacteria in turn, direct their defenses against these specific modes of action.

Understanding why antibiotics fail begins with the classification of antibiotics and their modes of action.

Antibiotic Classification

Grouped by Structure and Function

Five functional groups cover most antibiotics

1. Inhibitors of cell wall synthesis
2. Inhibitors of protein synthesis
3. Inhibitors of membrane function
4. Anti-metabolites
5. Inhibitors of nucleic acid synthesis

Antibiotics are usually classified based on their structure and/or function.

1. **Structure** - molecular structure.
   - ß-Lactams - Beta-lactam ring
   - Aminoglycosides - vary only by side chains attached to basic structure

2. **Function** - how the drug works, its mode of action.
   - 5 functional groups
   - These are all components or functions necessary for bacterial growth
   - Targets for antibiotics

In these discussions, we will primarily use the functional classification, but will point out where structural similarities also exist.

1. Inhibitors of Cell Wall Synthesis

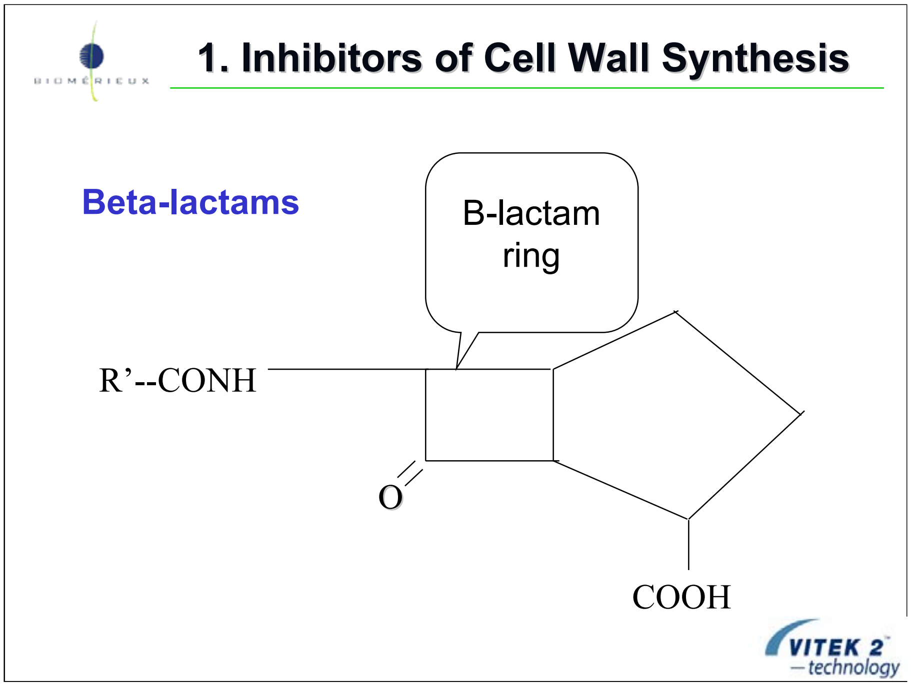
- **Beta-lactams**
  - Penicillins
  - Cephalosporins
  - Monobactams
  - Carbapenems

Glycopeptides
Fosfomycins

Inhibitors of Cell Wall Synthesis

Beta-lactams

- There are about 50 different Beta (ß)-lactams currently on the market
- They are all bactericidal
- They are non-toxic (i.e., they can be administered at high doses)
- They are relatively inexpensive
- Beta-lactams are organic acids and most are soluble in water



Inhibitors of Cell Wall Synthesis

Beta-lactams

- Similar in structure, as well as, function
- All have a common structural ß-lactam ring
- Antibiotics vary by side chains attached

- All beta-lactams are subject to inactivation by bacterial-produced enzymes called beta-lactamases

  Some more common Beta-lactamase enzymes include:

  - Penicillinases
  - Cephalosporinases
  - ESBL's
  - Cephamycinases
  - Carbapenemases

1. Inhibitors of Cell Wall Synthesis

Beta-lactams (Penicillins)	International Common Name
Penicillins (pen G)*	Penicillin G
Penicillinase-stable penicillins (pen M)	Oxacillin Methicillin
Aminopenicillins* (pen A)	Ampicillin Amoxicillin
Carboxypenicillins* (pen C)	Ticarcillin
Ureidopenicillins* (pen U)	Piperacillin
β-lactam / β-lactamase inhibitor combinations	Amoxicillin + clavulanic acid Ampicillin + sulbactam Ticarcillin + clavulanic acid Piperacillin + tazobactam
Amidinopenicillin	Mecillinam

* Penicillinase labile: hydrolyzed by staphylococcal penicillinase

Inhibitors of Cell Wall Synthesis

Beta-lactams - Penicillins

Spectrum of Action

1. Natural penicillins
   - **Penicillin G:** Active against Gram-positive organisms that do not produce beta-lactamases, *Neisseria* and some anaerobes
2. Penicillinase-resistant penicillins
   - **Penicillin M:** Active against penicillinase-producing *Staphylococci*
3. Extended-spectrum penicillins
   - **Aminopenicillins:** Slightly less active than Penicillin G against *Pneumococci, Streptococci and Meningococci*, but active against many strains of *Salmonella, Shigella,* and *P.mirabilis, H.influenzae*)
   - **Carboxypenicillins:** More stable than aminopenicillins to hydrolysis by the ß-lactamases of most *Enterobacteriaceae* and *Pseudomonas aeruginosa*
   - **Ureidopenicillins:** Greater activity than carboxypenicillins against Gram-positives, enterics and *P.aeruginosa*
4. Co-Drugs (Beta-lactam + beta-lactamase inhibitor)
   - **ß-lactamase inhibitors (BLI) combinations:** Additional activity against beta-lactamase producing organisms, including *Staphylococcus spp*., some enterics, *H.influenzae* and *Bacterioides spp*
5. Amidinopenicillins
   - **Mecillinam:** Restricted use to urinary infection with *E.coli*. Active against penicillinase and low-level cephalosporinase.

1. Inhibitors of Cell Wall Synthesis

Beta-lactamase Inhibitors (BLI)

Aim: to block β-lactamases

These enzymatic inhibitors have weak or poor antibacterial activity alone, but a strong affinity for β-lactamases: Clavulanic Acid
Sulbactam
Tazobactam

Never Alone!

Combination β-lactams - β-lactamase inhibitor:

Amoxicillin + Clavulanic Acid
Ampicillin + Sulbactam
Ticarcillin + Clavulanic Acid
Piperacillin + Tazobactam

Beta-lactamase inhibitors (BLI) have a beta(ß)-lactam ring, but have weak or poor antibacterial activity.

- They have a very high affinity for ß-lactamases
- They act as a trap, and are hydrolyzed in preference to the ß-lactam drug. The drug is left intact to act on the bacteria (cell wall).
- Should be called penicillinase inhibitors, because they are active against:
  - Staph penicillinase
  - Penicillinase of *K. pneumoniae*
  - ESBL (to a greater or lesser degree) - if the penicillinase is being overproduced, the inhibitor effect may be diluted (Inoculum Effect)

In the case of penicillinase production, where:

- Amoxicillin → R
- Amoxicillin + Clavulanic acid → usually S

- Inhibitors are active against all penicillinase (PASE and ESBL) but never on cephalosporinase

NEW ISSUE - BLI can act as inducers and actually stimulate enzyme (beta-lactamase) production. It is possible to see the following:

Pseudo monas	Ticarcillin = S	Ticarcillin/Clavulanic = R
Enterobacteriaceae	Piperacillin = S	Piperacillin/Tazobactam = R

1. Inhibitors of Cell Wall Synthesis

Beta-lactams (Cephems)	International Common Name
1st Generation Cephalosporins C1G	Cephalothin Cefazolin
2nd Generation Cephalosporins C2G	Cefuroxime Cefamandole
Cephamycin (new C2G)	Cefoxitin Cefotetan – removed
3rd Generation Cephalosporins C3G	Cefotaxime Ceftazidime Ceftriaxone
4th Generation Cephalosporins C4G	Cefepime
Oral C3G	Cefixime Cefpodoxime
Next Generation Cephalosporins	Ceftobiprole Ceftaroline

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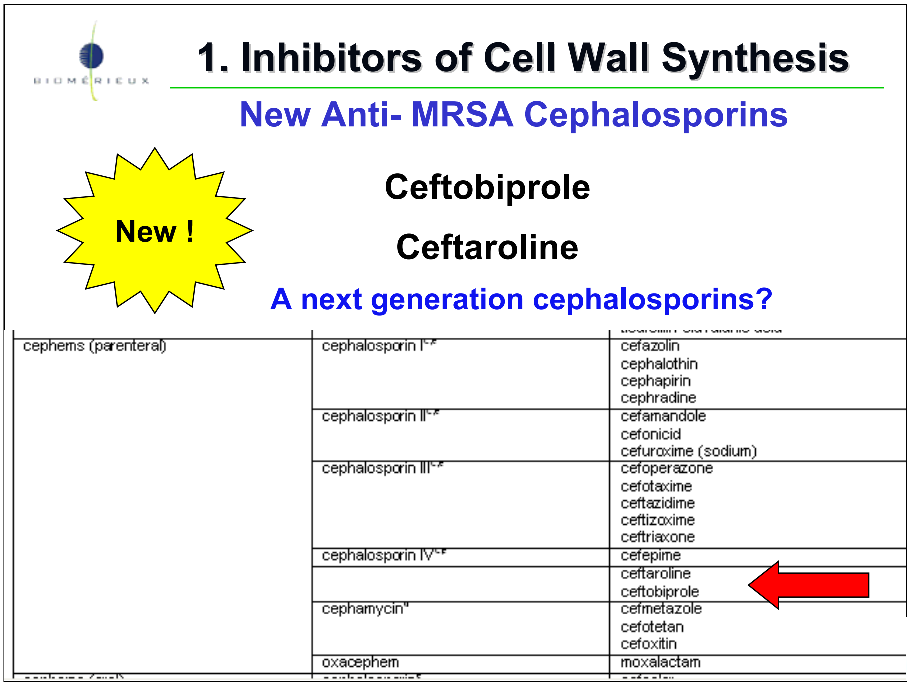
Inhibitors of Cell Wall Synthesis

Beta-lactams - Cephems

Spectrum of Action

- **1st generation cephalosporins (C1G):** Narrow spectrum; good Gram-positive activity and relatively modest Gram-negative activity. Inactivated by Gram-neg beta-lactamases (Derived from *Cephalosporium acremonium*).
- **2nd generation cephalosporins (C2G):** Better Gram-negative coverage (more beta-lactamase stability), but less *Staphylococcal* activity.
- **Cephamycins:** Remain susceptible in presence of Extended Spectrum ß-lactamase (ESBL) because they are not a substrate for the enzyme. Can be used as an indicator for ESBL (Derived from *Streptomyces lactamdurans*).
  - AstraZeneca discontinued its Cefotetan products in June 2006. Currently available from other manufacturers.
- **3rd generation cephalosporins (C3G):** Wider spectrum of action when compared to C1G and C2G. Less active than narrow spectrum agents against Gram-positive cocci, but much more active against the *Enterobacteriaceae* and *Pseudomonas aeruginosa* (better beta-lactamase stability).
- **4th generation cephalosporins (C4G):** Broadest spectrum of action. Active against high level cephalosporinases of *Enterobacteriaceae* and *Pseudomonas aeruginosa* (not usually used with ESBL producing organisms).

None have activity to MRSA or *Enterococcus spp*.



cephems (parenteral)	cephalosporin I[c,#]	cefazolin cephalothin cephapirin cephradine
	cephalosporin II[c,#]	cefamandole cefonicid cefuroxime (sodium)
	cephalosporin III[c,#]	cefoperazone cefotaxime ceftazidime ceftizoxime ceftriaxone
	cephalosporin IV[c,#]	cefepime
		ceftaroline ceftobiprole
	cephamycin[#]	cefmetazole cefotetan cefoxitin
	oxacephem	moxalactam

Inhibitors of Cell Wall Synthesis

Beta-lactams - Cephems - Ceftobiprole

Spectrum of Action

- **Next generation cephalosporin:** Broad spectrum; active against the common Gram-negative bacteria. Some Gram-positive activity (Drug Resistant *S. pneumoniae*). Notable for activity against MRSA, unlike any other beta-lactam antibiotic. Bactericidal.

- Not yet FDA approved
- Has been added to CLSI® M100-S18, 2008 listing of antibiotics
- No CLSI breakpoints
- Once FDA approved, can no longer say if Staph is resistant to Oxacillin, report **all** beta-lactams as resistant.

1. Inhibitors of Cell Wall Synthesis

Beta-lactams	International Common Name
Monobactams	Aztreonam
Penems	
Carbapenems	Imipenem Meropenem Ertapenem Doripenem
Penems	Faropenem

VITEK 2 technology

Inhibitors of Cell Wall Synthesis

Beta-lactams: Monobactams

Spectrum of Action

- **Aztreonam:** Gram-negatives (*Enterobacteriaceae* and *Pseudomonas*). Not hydrolyzed by most commonly occurring plasmid and chromosomally mediated ß-lactamases, and does not induce the production of these enzymes.

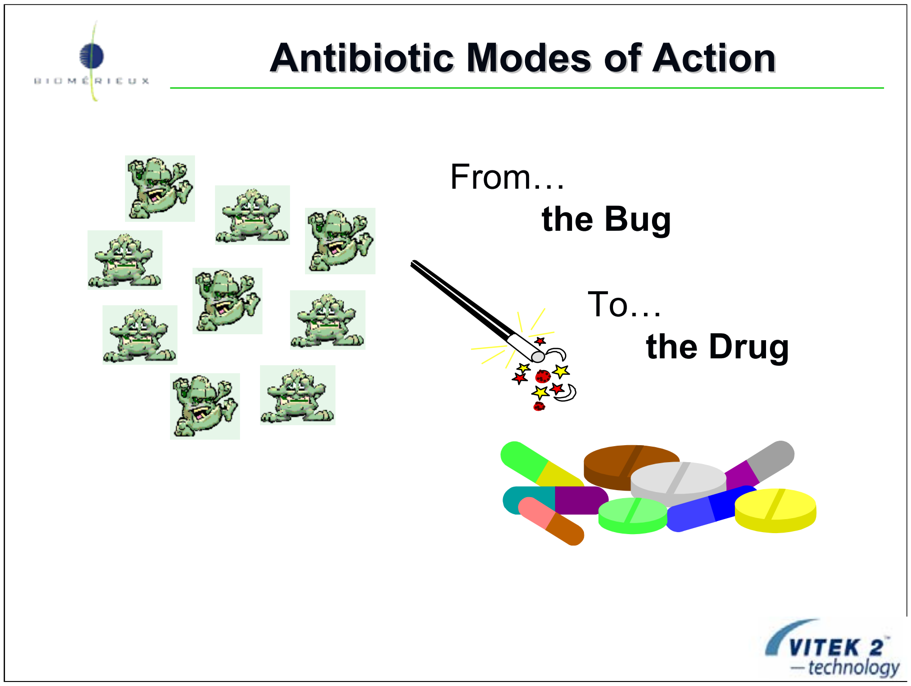
Beta-lactams: Penems

Slightly different structure than the other ß-lactams, make the Penems much more resistant to beta-lactamase hydrolysis.

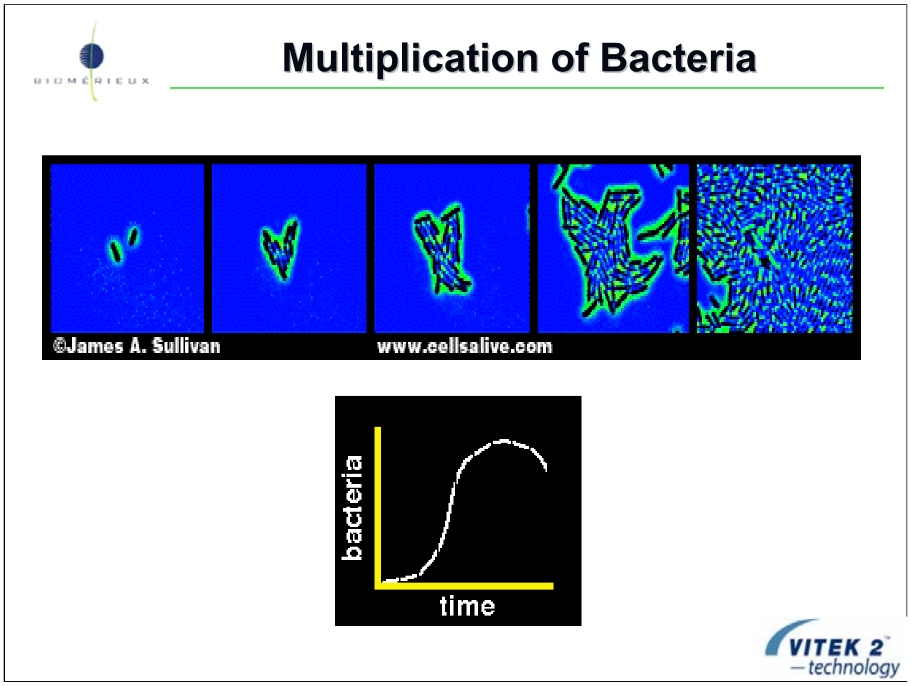
Spectrum of Action

- **Carbapenems:** ß-lactams with a broad spectrum of action. Gram-positives, except MRSA. Gram-negatives, *Ps. aeruginosa* (except Ertapenem) and anaerobes. Very efficient against high level cephalosporinase and ESBL. Wide diffusion in the body, especially in the cerebrospinal fluid.
  Doripenem (new) - Broad spectrum. Particularly good for *Pseudomonas* and other non-fermenters.

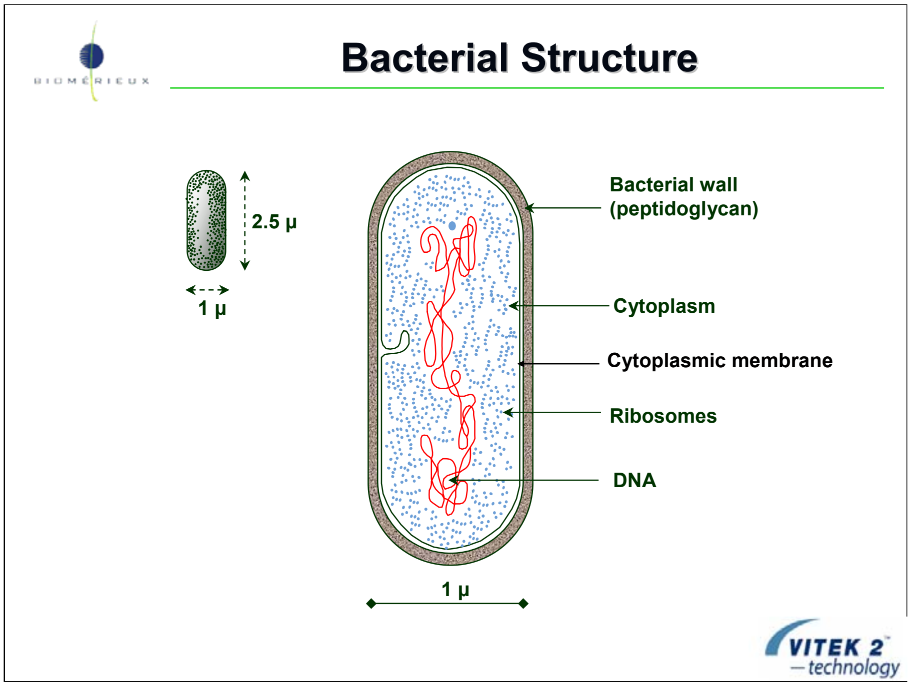
- **Penem:** Oral. Primarily for respiratory tract infections. Poor activity with *Serratia, Pseudomonas, Stenotrophomonas*.
  Faropenem not yet FDA approved.



- Before we can understand the antibiotic modes of action, we need to review the structure and physiology of the bacterial cell



- It is important to understand the growth cycle and physiology of bacteria to appreciate how it influences antimicrobial action
- Bacteria are all around us. Given good growing conditions, a bacterium will divide.
- A new cell wall forms at the center and the "bug" splits into two daughter cells, both of which carry the same genetic information as the parent
- If the environment is perfect, the two daughter cells can split into four within 20 minutes
- 1, 2, 4, 8, 16, 32, 64...
- Typical bacterial growth cycle includes:
  - Lag phase
  - Exponential (log) phase
  - Stationary phase
  - Death phase



Typical Structure of a Bacterial Cell (from inside to outside)

DNA bacterial genetic material

Ribosomes (protein-making factories), energy-generating systems, digestive system, and everything else are located in the cytoplasm.

Cytoplasmic Membrane or Inner Membrane

a. Consists of phospholipids and other membrane proteins

b. Semi-permeable

c. Regulates pH, osmotic pressure and availability of essential nutrients

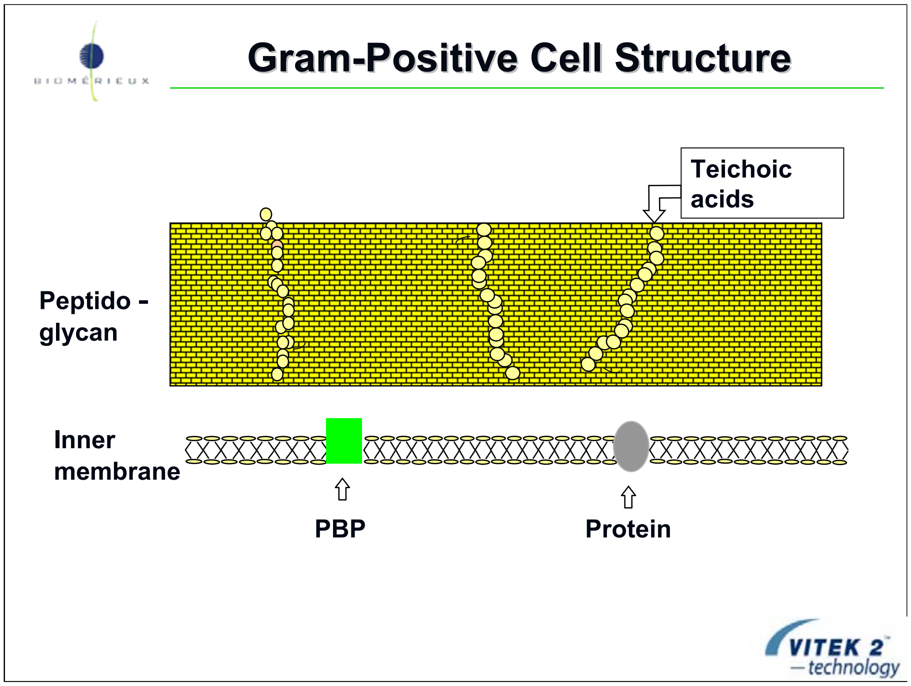
Bacterial Cell Wall or Peptidoglycan

a. Cross-linked mesh that gives a cell its shape, strength and osmotic stability, a protective suit of armour

b. Porous up to 100,000 Da

The outer layer of lipopolysaccharide (LPS) and phospholipid material helps protect bacteria from bacteriophages, pH, enzymes, phagocytosis.

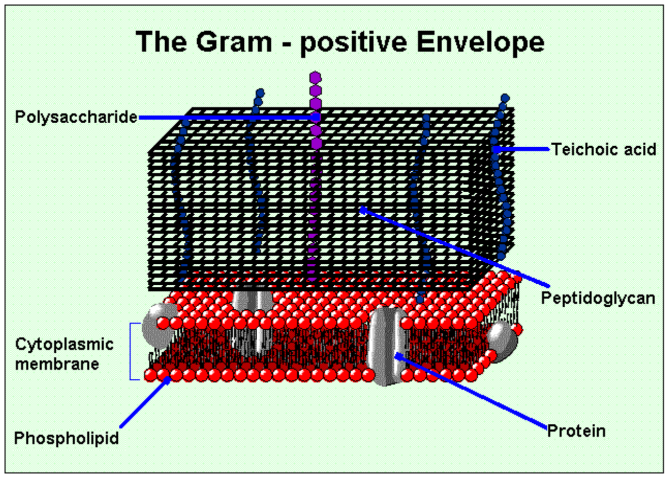
- To multiply, the bacteria must be able to synthesize peptidoglycan, proteins and DNA
- The cell wall, the ribosomes and DNA are all potential antibiotic targets

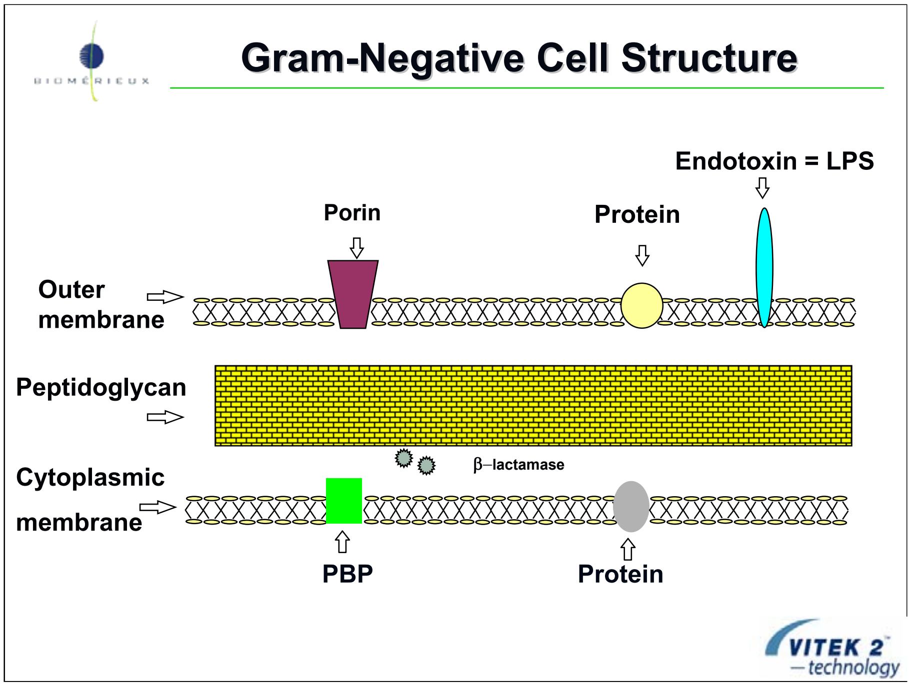


Gram-Positive Cell Structure

- The Gram-positive cell wall is **thick** and consists of 90% **peptidoglycan**
- **Teichoic acids** link various layers of peptidoglycan together. Teichoic acids also regulate the autolysin activity in this complex equilibrium.
- The **cytoplasmic membrane** (which defines the intracellular space) consist of:
  - a **lipid bilayer**
  - **intrinsic proteins** which are hydrophobic (mostly enzymes involved in respiration and transmembrane transport)
  - **extrinsic proteins** which are hydrophilic
  - **Penicillin-Binding Proteins (PBPs)**: periplasmic space proteins involved in peptidoglycan synthesis (glycosyltransferase, transpeptidase and carboxypeptidase activities)

Gram-Positive Cell Structure

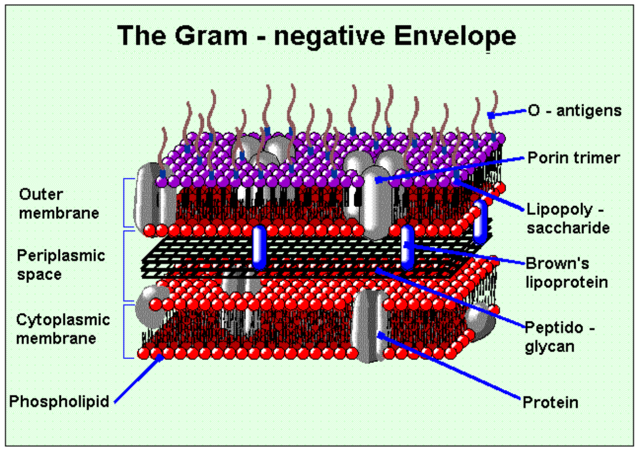


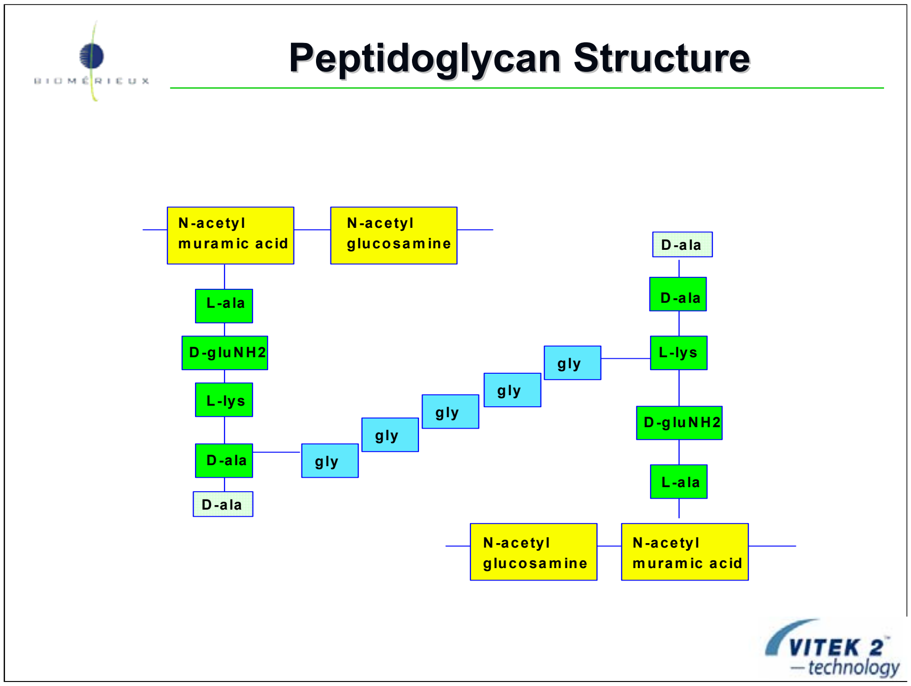


Gram-Negative Cell Structure

- The **outer membrane** is made up of:
  - **phospholipids**
  - endotoxin or **lipopolysaccharide (LPS)** - plays an important role in the antibiotic entry into the cell
  - **proteins** including the **porins** (complexes of three proteins) form aqueous channels that provide a route across the outer membrane for all the water-soluble compounds needed by the bacterium
- The **periplasmic space** contains:
  - **peptidoglycan** – 5-20% of cell wall
  - various enzymes (in particular, **ß-lactamases**)
- The **cytoplasmic membrane** (which defines the intracellular space) consists of:
  - a **lipid bilayer**
  - **intrinsic proteins** which are hydrophobic (mostly enzymes involved in respiration and transmembrane transport)
  - **extrinsic proteins** which are hydrophilic
  - **Penicillin-Binding Proteins (PBPs)** - periplasmic space proteins involved in peptidoglycan synthesis (glycosyltransferase, transpeptidase and carboxypeptidase activities)

Gram-Negative Cell Wall Structure



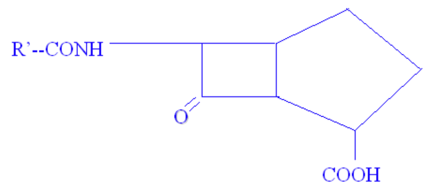


Peptidoglycan

- is a very high molecular weight polymer composed of many identical subunits
- is a 3-D polymeric macromolecule
- determines cell shape and prevents osmotic lysis, porous up to 100,000 daltons
- contains N-acetylglucosamine (NAG), N-acetylmuramic acid (NAMA), and several different amino acids
- involves 2 types of covalent chemical bonds:
    1. B-1, 4 glycosidic bond between hexose sugars
    2. Petide bond between amino acids
- In Gram-positive bacteria, peptidoglycan accounts for as much as 90% of the cell wall (approximately 40 layers), with the rest consisting of the *teichoic acids*.
- Gram-negative bacteria have a thin peptidoglycan layer (accounting for only 5-20% of the cell wall). However, in addition to the cytoplasmic membrane, they have a second phospholipid bilayer external to the peptidoglycan called the outer membrane.

1. Inhibitors of Cell Wall Synthesis

Mode of Action
of
Beta-lactams



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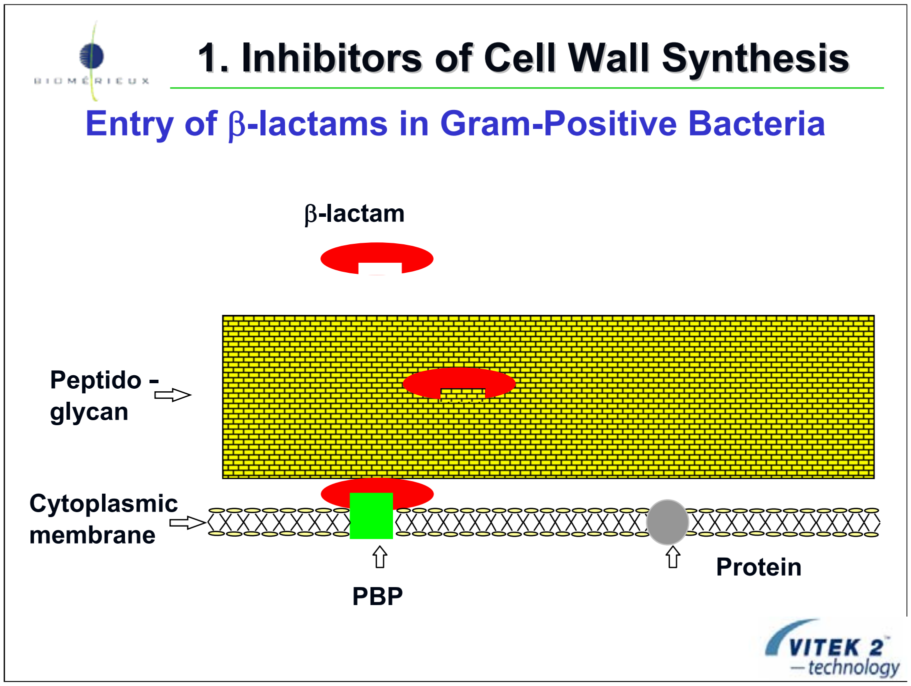
Humans have no cell wall (no peptioglycan), so this is a good selective target for the antibiotic.



Beta-lactams are mostly water soluble.

Differences in cell wall composition (more or less lipids) between different bacterial species partially account for their differential susceptibility to ß-lactams.

The **target** of the ß-lactam antibiotics for all bacteria is the **PBP** (Penicillin Binding Protein) in the cytoplasmic membrane.

<u>Gram-Positives</u>:

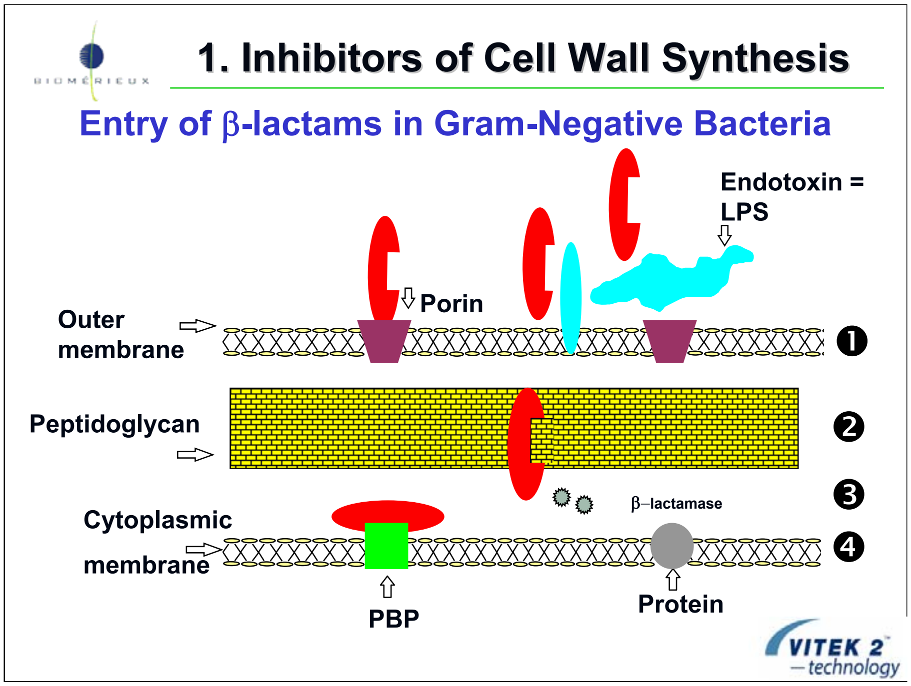
In Gram-positive bacteria, there is no barrier to the entry of ß-lactams antibiotics.

The peptidogylcan layers allow the diffusion of small molecules.

However, **ß-lactams cross the membranes with great difficulty due to high lipid** content but, since their target PBP's are found on the outer surface of the cytoplasmic membrane, they only have to cross the cell wall.

Natural Resistance

Enterococcus - PBP's are different from other Gram-positives (also higher lipid content in cell wall), which causes a low level resistance to penicillins and resistance to C1G.



Gram-Negatives:

1. Outer membrane entry through the:

   Porins: Hydrophilic ß-lactams tend to gain access into the periplasmic space using these watery funnels (i.e., *E. coli* organism has about 100,000 of these porins). Porins are transmembrane proteins. They complex together to form water-filled channels through which low molecular weight (<600 daltons) hydrophilic substances readily diffuse. Such diffusion is passive and the concentration in the periplasmic space can reach the level that prevails in the external environment as long as there are no mechanisms to pump the drug back out or which inactivate it.

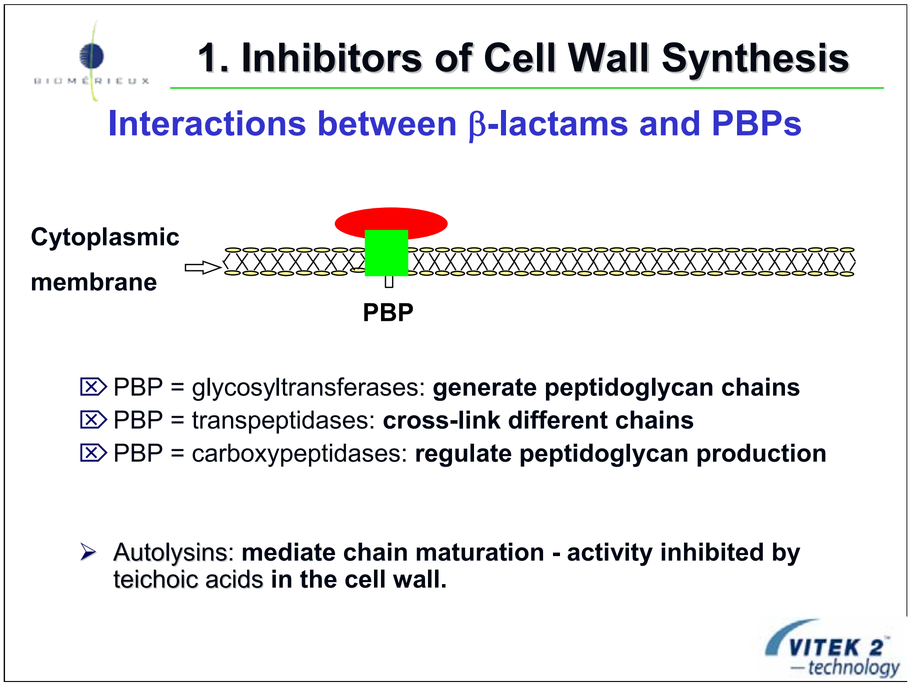
   Phospholipids: This mode of entry is less common, but it seems to play a significant part in the case of certain ß-lactams. Lipid bilayers support the **diffusion** of lipophilic compounds (certain ß-lactams are lipophilic). However, if the organism's LPS (endotoxin) is branched (e.g., as in the case of *Pseudomonas aeruginosa*), such diffusion is blocked. This is called impermeability.

2. Peptidoglycan entry

3. Periplasmic space entry: ß-lactams may encounter ß-lactamases

4. Cytoplasmic membrane PBP - bound

Natural Resistance - Many Gram-negative organisms are naturally resistant to penicillin G and oxacillin because the drug is prevented from entering the cell by the LPS which blocks the porins.

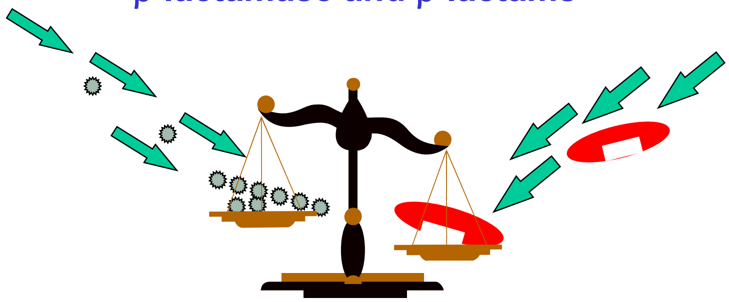


- PBPs are enzymes involved in peptidoglycan synthesis (glycosyltransferases, transpeptidases and carboxypeptidases)
- There are many kinds of PBP's - some essential, some not
- They are numbered by molecular weight
- Inhibition of these enzymes by ß-lactams inhibits peptidoglycan synthesis and therefore stops cell growth (bacteriostatic activity)
- ß-lactams form stable complexes with PBPs
  - Bind irreversibly to PBP by a covalent bond
- ß-lactams also have bactericidal activity. Although peptidoglycan synthesis is stopped, the autolysins remain active. Autolysin activity is progressively potentiated. The peptidoglycan network begins to become disorganized and teichoic acid (which normally regulates autolysin activity by natural inhibition) tends to leak out.
- Without a peptidoglycan layer, the bacterium bursts and eventually all cells die

☒ **NOTE:** Ceftobiprole and Ceftaroline (the next generation cephalosporins) have the ability to inactivate PBP2a which is primarily responsible for oxacillin resistance in *Staphylococci.*

1. Inhibitors of Cell Wall Synthesis

β-lactamase and β-lactams



The efficacy of the antibiotic hangs on 2 parameters:

- The rapidity of the drug entrance
- The rate of enzymatic hydrolysis

Periplasmic Space Entry

- The periplasmic space represents a “mine field” for ß-lactams due to the presence of ß-lactamase enzymes (defense enzymes)
- ß-lactamases are found in all bacteria, although in variable amounts and with varying levels of activity (they can even be found in wild-type *E. coli* although at such a low concentration that their effect is insignificant).
- ß-lactamases hydrolyze the ß-lactam ring. The rate of this hydrolysis depends on the rate on entry of the drug and the level of ß-lactamase activity.
- In many cases, the rate of entry is sufficient to guarantee a consistently high enough concentration of the drug to interact with the PBP’s, even if a certain proportion is constantly being enzymatically hydrolyzed.

1. Inhibitors of Cell Wall Synthesis

Beta-lactams
- Penicillins
- Cephalosporins
- Monobactams
- Carbapenems

➢ **Glycopeptides**

Fosfomycins

VITEK 2 technology

Inhibitors of Cell Wall Synthesis

Glycopeptides

- The term 'Glycopeptide' included two main compounds with very similar structures and modes of action:

  Vancomycin and Teicoplanin

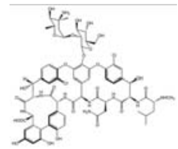
- Teicoplanin not FDA approved in the U.S.
- Both are of high molecular weight (1500-2000 daltons)
- Glycopeptides have a complex chemical structure
- Inhibit cell wall synthesis at a site different than the beta-lactams
- All are bactericidal
- All used for Gram-positive infections. (No Gram-negative activity)
- Pharmaceutical research and development has been very active in this area recently resulting in new antimicrobials and classification

1. Inhibitors of Cell Wall Synthesis

Glycopeptides

Glycopeptide:

Vancomycin →



Lipoglycopeptide:

Dalbavancin

Oritavancin

Telavancin

Teicoplanin

New

Inhibitors of Cell Wall Synthesis

Glycopeptides: Glycopeptide

Spectrum of Action

- **Vancomycin:** MRSA (Methicillin Resistant *Staph. aureus*), *C.difficile, Streptococci* including *Strep pneumoniae.* Alternative to Penicillin G in serious infections. Good diffusion in all tissues (except CSF). High toxic effects: ears and kidneys.

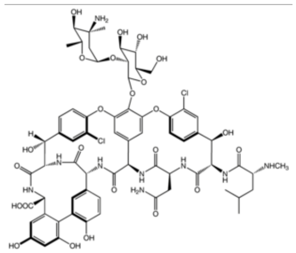
Glycopeptides: Lipoglycopeptide

Spectrum of Action

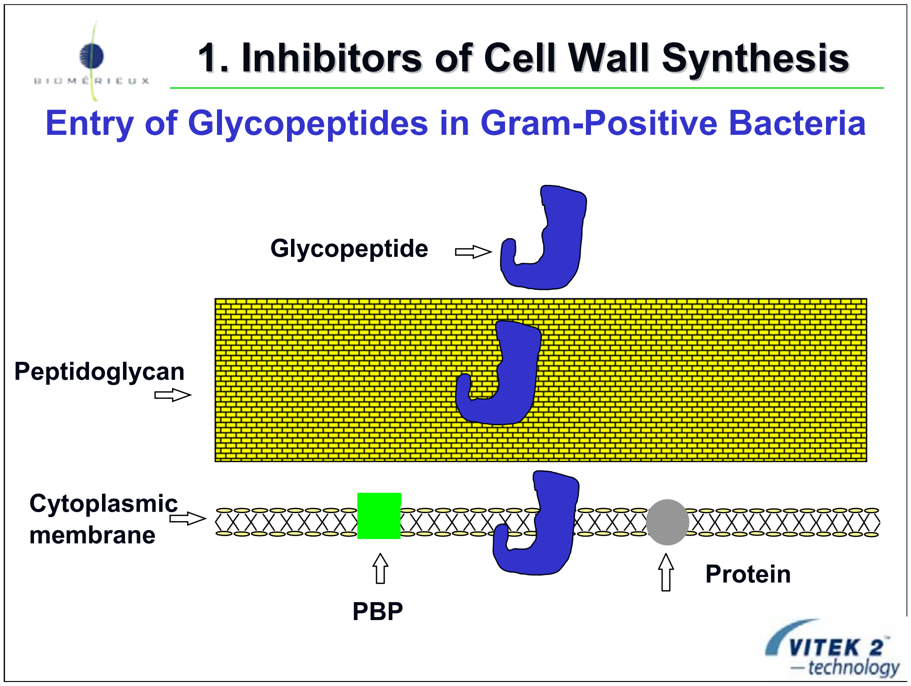
- **Dalbavancin:** (Vicuron) 2nd generation lipoglycopeptide. Bactericidal for MRSA and MRSE.1/week IV dosing – long acting. Not yet FDA approved.
- **Oritavancin:** (Targanta) **2nd generation glycopeptide**. Actually a lipoglycopeptide. IV – once per day dosing. For skin and skin structure infections. Activity similar to Vancomycin – better for *Staphyloccus* and *Enterococcus*. Not yet FDA approved. Bactericidal
- **Telavancin:** (Theravance) bactericidal for all Gram-positive. Inhibits cell wall synthesis and inhibits bacterial phospholipid membrane synthesis. Single dose injectable. Not yet FDA approved.
- **Teicoplanin:** Not FDA approved in the US. Widespread use in Europe.

1. Inhibitors of Cell Wall Synthesis

Mode of Action of Glycopeptides

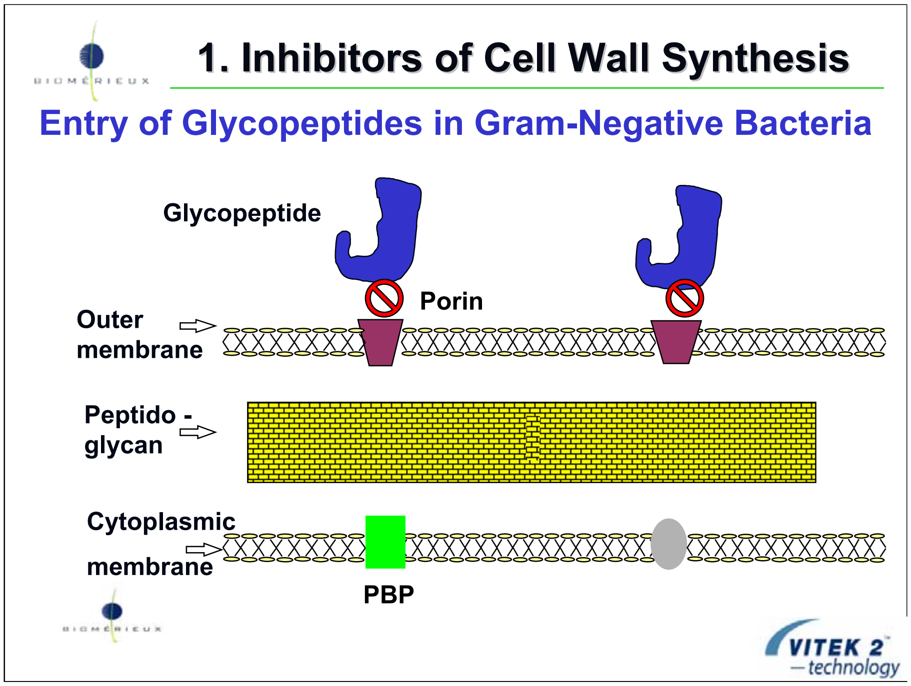


Vancomycin



In Gram-Positives:

The drugs enter without any problem because peptidoglycan does not act as a barrier for the diffusion of these molecules.



In Gram-Negatives:

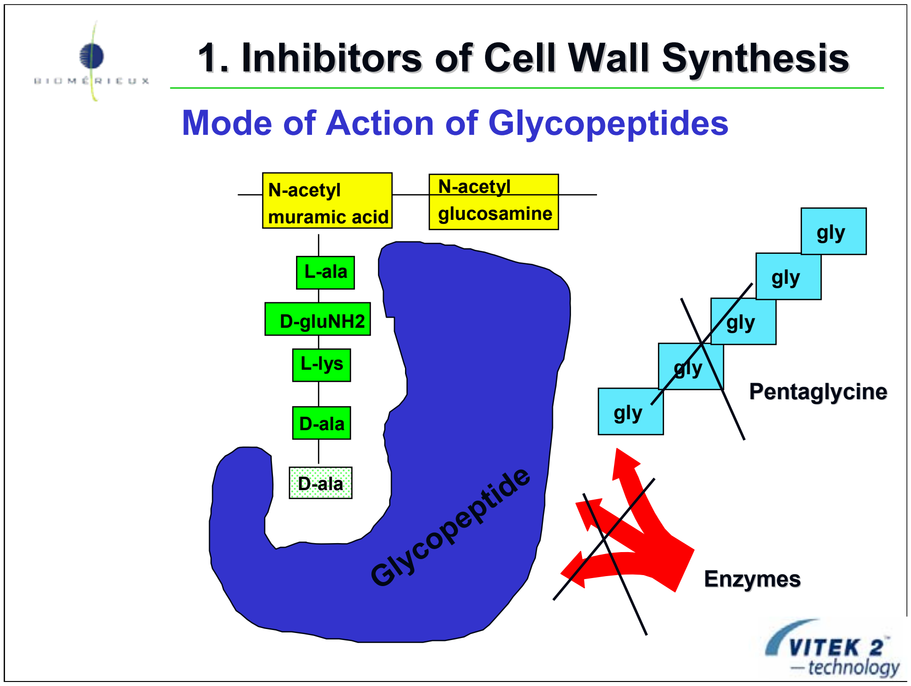
Glycopeptides are of high molecular weight (1500-2000 daltons), stopping them from passing through the porins of gram-negative bacteria (i.e., glycopeptides have no activity against Gram-negatives).

Gram-negatives are naturally resistant.

Use this property in Microbiology in several ways:

Check Gram reaction - growth around Vancomycin disk would indicate a Gram-negative organism (resistant to Vancomycin).

KVC disks/media - for anaerobe ID’s.



- Glycopeptides inhibit the final cell wall stage of the peptidoglycan synthesis process
- The 'pocket-shaped' glycopeptide binds the **D-ala-D-ala** terminal of the basic sub-unit theoretically waiting to be incorporated into the growing peptidoglycan
- Because it is so bulky, the glycopeptide inhibits the action of the glycosyltransferases and transpeptidases (which act as a kind of "cement") - blocks pentaglycine from joining molecules, thereby blocking peptidoglycan growth.
- Glycopeptides are bactericidal, but slow-acting

1. Inhibitors of Cell Wall Synthesis

Beta-lactams
- Penicillins
- Cephalosporins
- Monobactams
- Carbapenems

Glycopeptides

➢ **Fosfomycins**

Inhibitors of Cell Wall Synthesis

Fosfomycins

Spectrum of Action

Fosfomycin: Acts to inhibit cell wall synthesis at a stage earlier than the penicillins or cephalosporins. FDA approved 1996. While it is a broad spectrum agent, CLSI® only provides breakpoints for urinary tract infections due to *E.coli* and *E.faecalis*.

Reference Method:

Preferred reference method is agar dilution. Broth dilution should not be performed.

Mode of Action:

Inhibits the first step of the peptidoglycan synthesis process

2. Inhibitors of Protein Synthesis

Aminoglycosides

MLSK
(Macrolides, Lincosamides, Streptogramins, Ketolides)

Tetracyclines

Glycylcyclines

Phenicols

Oxazolidinones

Ansamycins

VITEK 2 technology

Inhibitors of Protein Synthesis

Aminoglycosides - (Bactericidal)	Gentamicin Tobramycin Amikacin
MLSK - (Bacteriostatic)	Erythromycin Clindamycin Quinupristin-Dalfopristin (Synercid) Clarithromycin Azithromycin Telithromycin
Tetracyclines - (Bacteriostatic)	Tetracycline Doxycycline Minocycline
Glycylcyclines -	Tigecycline
Phenocols - (Bacteriostatic)	Chloramphenicol
Oxazolidinones -	Linezolid

(Bactericidal for *Streptococci*; Bacteriostatic for *Enterococcus* and *Staphylococci*)

Ansamycins -	Rifampin

(Bacteriostatic or Bactericidal depending on organism and concentration)

2. Inhibitors of Protein Synthesis

- **Aminoglycosides**

MLSK
(Macrolides, Lincosamides, Streptogramins, Ketolides)

Tetracyclines

Glycylcyclines

Phenicols

Oxazolidinones

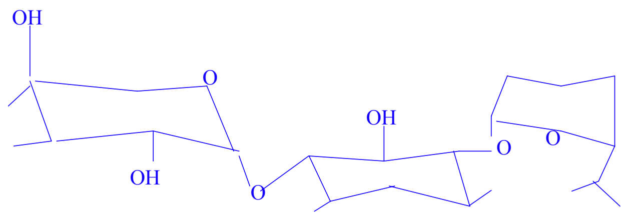
Ansamycins

Aminoglycosides

- Humans do synthesize proteins, but at a much slower rate than bacteria
- Drug can act on bacteria before it does damage to man
- But, since it is a common process, we do tend to see more toxic side effects
- Need to do therapeutic drug monitoring
- All bactericidal

2. Inhibitors of Protein Synthesis

Aminoglycosides



VITEK 2 technology

Inhibitors of Protein Synthesis

Aminoglycosides:

- Related in structure and function
- Drugs differ based on location of radical groups attached to the 3 ring basic structure

2. Inhibitors of Protein Synthesis

Aminoglycosides

AMINOGLYCOSIDES	International Common Name
	Amikacin Gentamicin Tobramycin Streptomycin Kanamycin Netilmicin

Inhibitors of Protein Synthesis

Aminoglycosides:

Spectrum of Action

- Rapid bactericidal effect
- Broad spectrum of action

  Gram-negative nosocomial infections and severe systemic infections

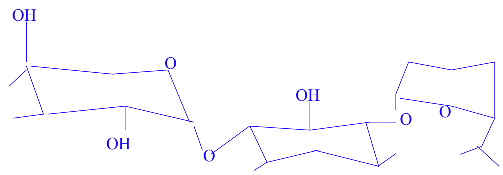
  Gram-positives, except *Streptococus* and *Enterococcus*. Must combine an aminoglycoside (Gentamicin or Streptomycin) with a penicillin, ampicillin or vancomycin for severe enterococcal infections (Synergy Testing).
- In serious infection, used in association with beta-lactams or fluoroquinolones
- Kanamycin develops resistance quickly
- Hospital use only
- Nephrotoxic and toxic for ears

Drug Dosage Adjustment:

Monitoring aminoglycosides is mandatory. It is important to control the serum level for peak and trough to ensure the bactericidal effect and avoid side effects.

2. Inhibitors of Protein Synthesis

Mode of Action
of
Aminoglycosides



2. Inhibitors of Protein Synthesis

Protein Synthesis

A quick review of protein synthesis before we begin.

2. Inhibitors of Protein Synthesis

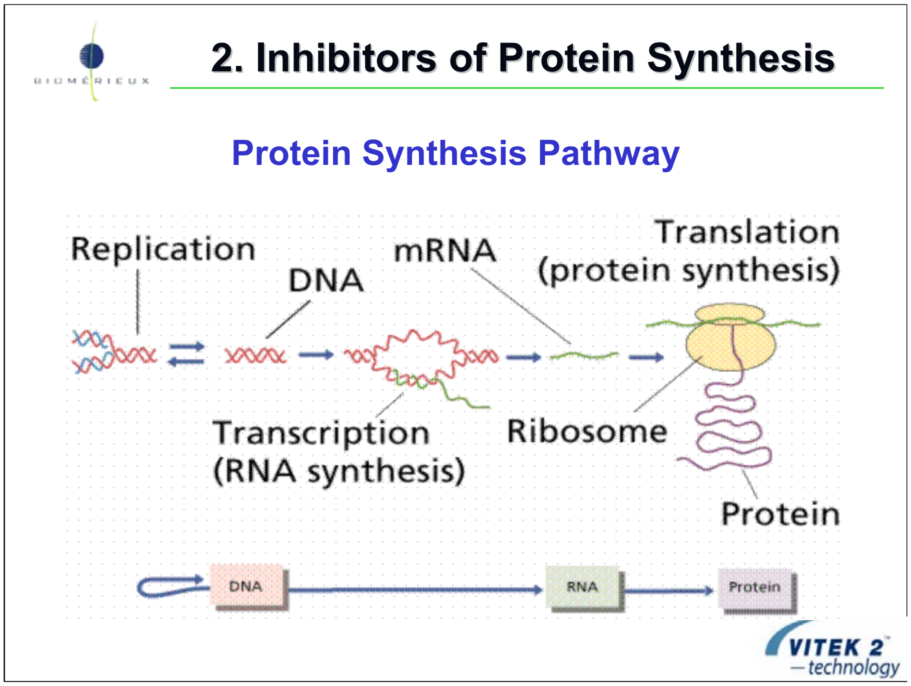
Protein Synthesis

Proteins are made up of at least 20 different amino acids

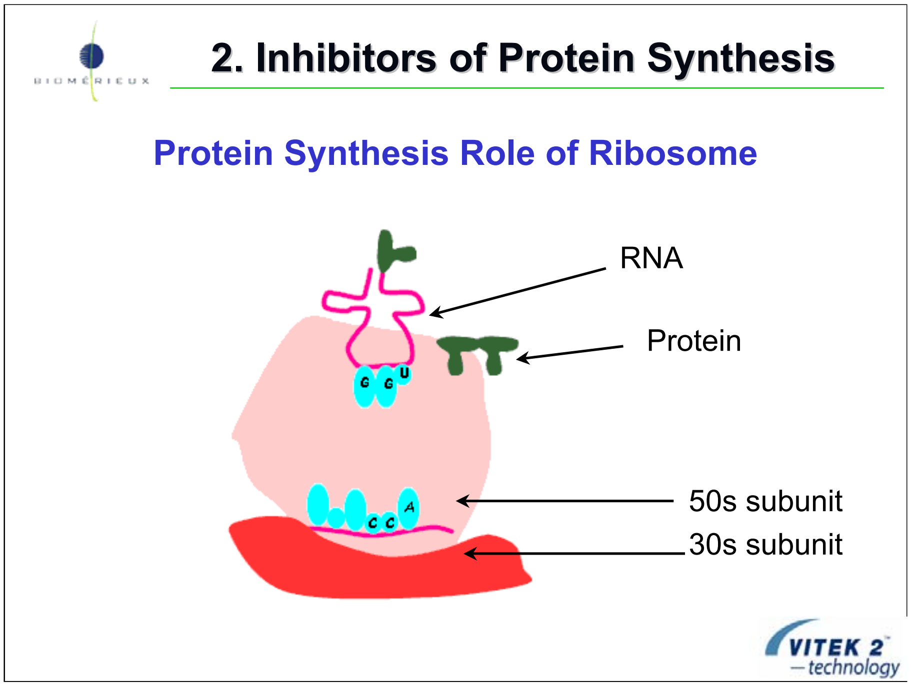
Thousands of different proteins exist, each with its own function (enzymes, etc.).

Each protein is manufactured in the cell using a code contained in the DNA.

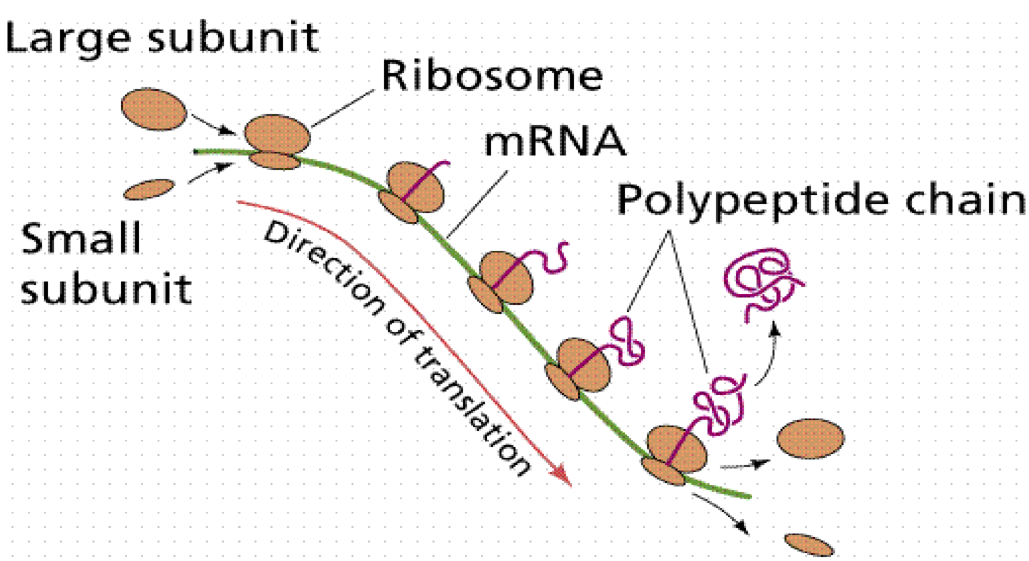
- The code for the 20 essential amino acids consists of at least a 3-base set (triplet) of the 4 bases
- If one considers the possibilities of arranging four things 3 at a time (4X4X4), we get 64 possible code words, or codons (a 3-base sequence on the mRNA that codes for either a specific amino acid or a control sequence).

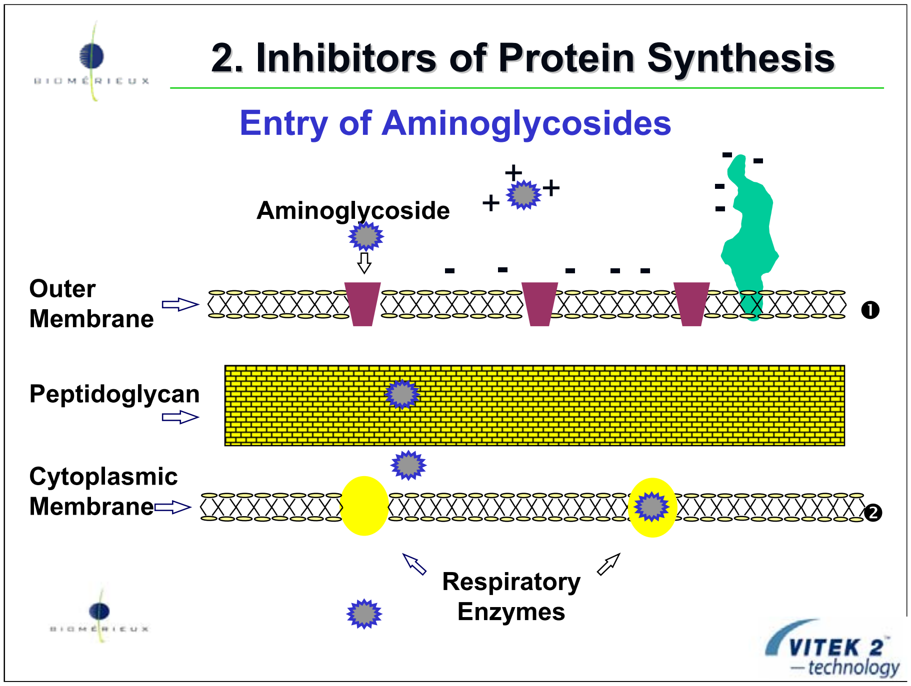


- Proteins are manufactured in the cell using a code in the DNA
- DNA is transcribed into RNA by an enzyme called RNA polymerase
- The RNA strand is translated into proteins by the ribosomes



- Each ribosome consists of one small sub-unit (30S) and a larger one (50S)
- The ribosome crawls along the messenger-RNA molecule translating the code and assembling the amino acids in the correct order before chemically joining them to create the protein





Aminoglycoside Mode of Action

Target = Ribosome in cytoplasm

1. Outer Membrane entry:

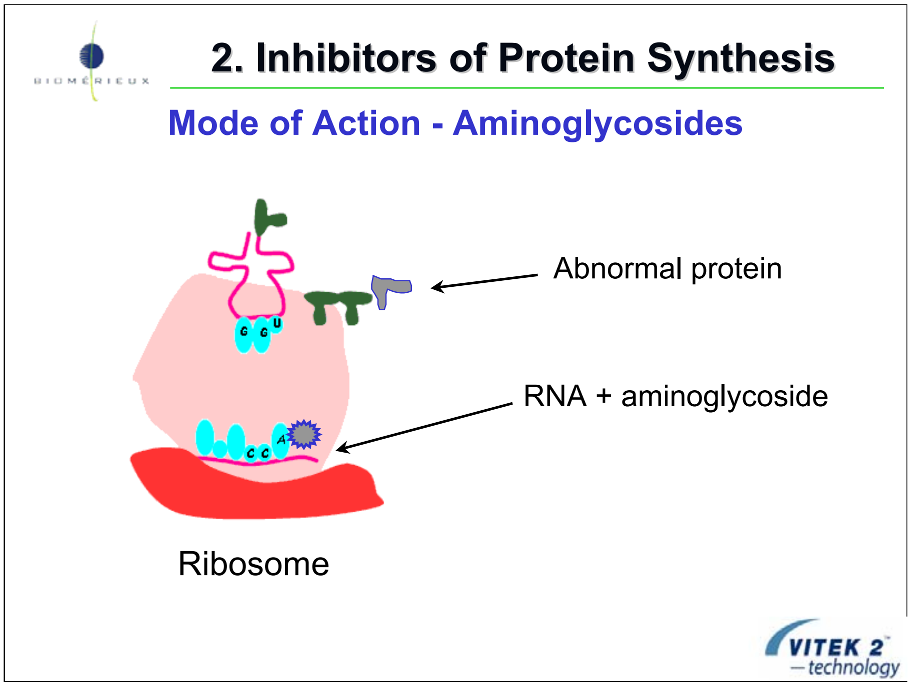
   Aminoglycosides are positively charged molecules which means they rapidly enter bacteria (negatively charged) since the two charges attract each other.

   The negative charge of bacteria is due to LPS in the outer membrane and the peptidoglycan (notably the teichoic acid).

2. Cytoplasmic Membrane entry:

   The drugs cross the cytoplasmic membrane via respiratory enzymes (involved in aerobic respiration).

   This is why bacteria without respiratory enzymes (strict anaerobes or facultative anaerobes like streptococci) are **naturally resistant** to aminoglycosides.



Aminoglycosides bind to the RNA of the 30S ribosomal sub-unit.

The resulting change in ribosome structure affects all stages of normal protein synthesis.

- Initiation step of translation
- Blocks elongation of peptide bond formation
- Release of incomplete, toxic proteins

Translational errors are frequent and **many non-functional or toxic proteins** are produced. The incorporation of such abnormal proteins into the cytoplasmic membrane compromises its function.

The bactericidal activity of aminoglycosides ultimately stops protein synthesis and dramatically damages the cytoplasmic membrane.

2. Inhibitors of Protein Synthesis

Aminoglycosides

➢ MLSK
(Macrolides, Lincosamides, Streptogramins, Ketolides)

Tetracyclines

Glycylcyclines

Phenicols

Oxazolidinones

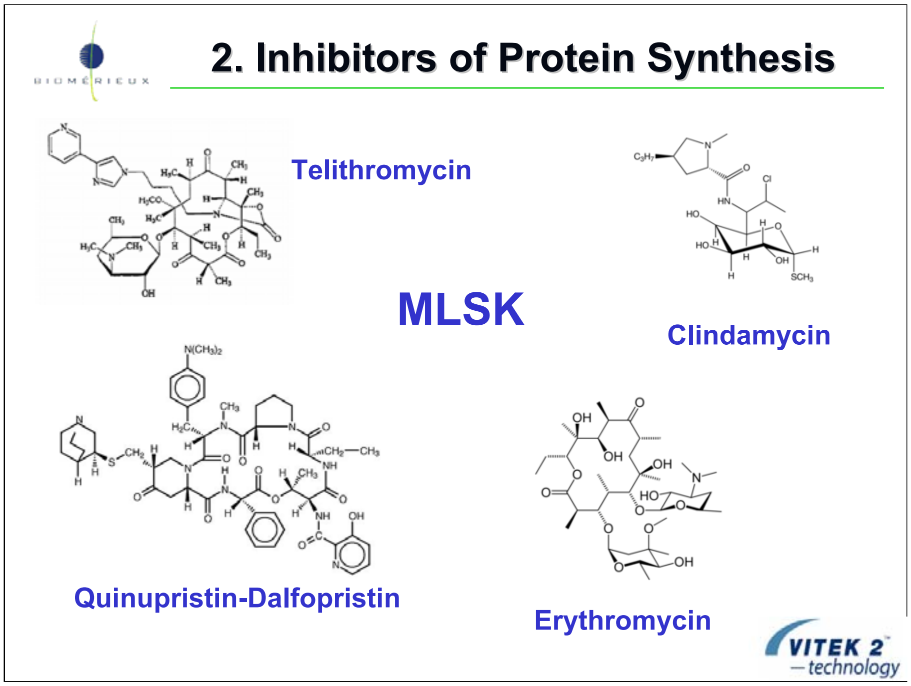
Ansamycins

Inhibitors of Protein Synthesis

MLSK: (Macrolides, Lincosamides, Streptogramins, Ketolides)

Bacteriostatic

Their spectrum of activity is limited to Gram-positive cocci such as *Streptococci* and *Staphylococci*. These antibiotics are also active against anaerobes.



Inhibitors of Protein Synthesis

MLSK: (Macrolides, Lincosamides, Streptogramins, Ketolides)

Four different classes of antibiotics which are unrelated in terms of structure but which have a similar mode of action and spectrum of activity.

2. Inhibitors of Protein Synthesis

Macrolides, Lincosamides, Streptogramins, Ketolides MLSK

	International Common Name
Macrolides	Erythromycin
Lincosamides	Lincomycin Clindamycin
Streptogramins	Quinupristin / Dalfopristin Pristinamycin
Ketolides	Telithromycin

NEW

Inhibitors of Protein Synthesis

Spectrum of Action

Macrolides: Respiratory infections due to *S. pneumoniae* and *S. pyogenes*, *Mycoplasma, Legionella*, less serious *Staphylococcal* infections.

Lincosamides: Gram-positive skin infections and anaerobe infections. Oral administration appropriate for out patient settings.

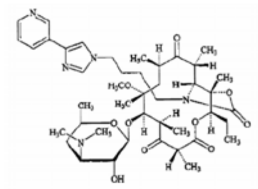
Streptogramins: Quinupristin/Dalfopristin (Synercid): consists of an A and B component (Synergistic).

> **A** - prevents peptide bond formation and changes the ribosome to attract the B component.
>
> **B** - causes early release of incomplete peptide chains. Used for *E. faecalis* (VRE) and MRSA.

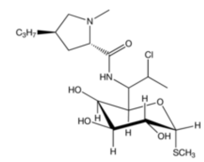
Ketolides: Telithromycin: Represents a novel class that has received much attention recently due to their excellent activity against resistant organisms.

> Ketolides are semi-synthetic derivatives of erythromycin.
>
> FDA approved for bronchitis, sinusitis, community acquired pneumonia due to *S.pneumoniae* (including Macrolide Resistant strains), *H. influenza, M.catarrhalis, S.aureus* (sinusitis), *M.pneumoniae,* and *C.pneumoniae*
>
> Side effects – possible liver damage.

2. Inhibitors of Protein Synthesis

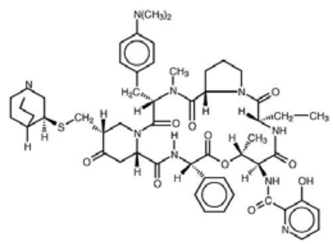


Telithromycin

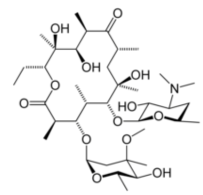


Clindamycin

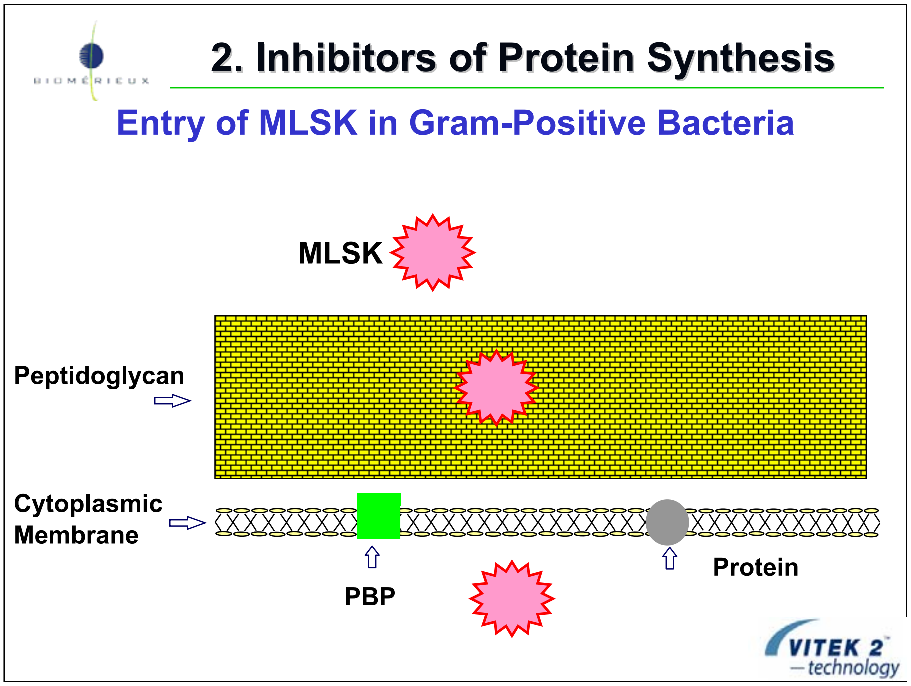
Mode of Action of MLSK



Quinupristin-Dalfopristin

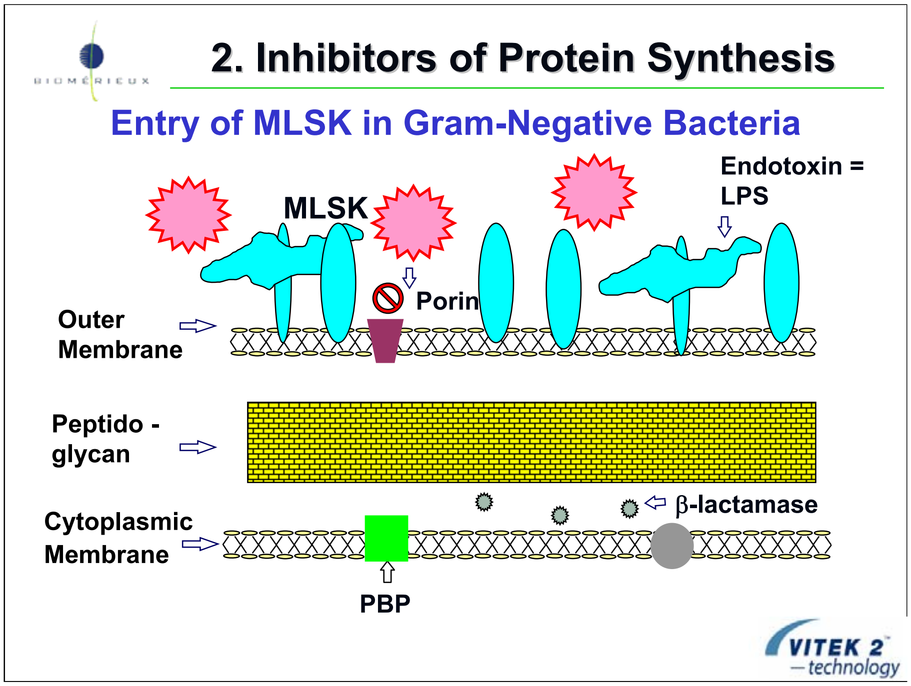


Erythromycin

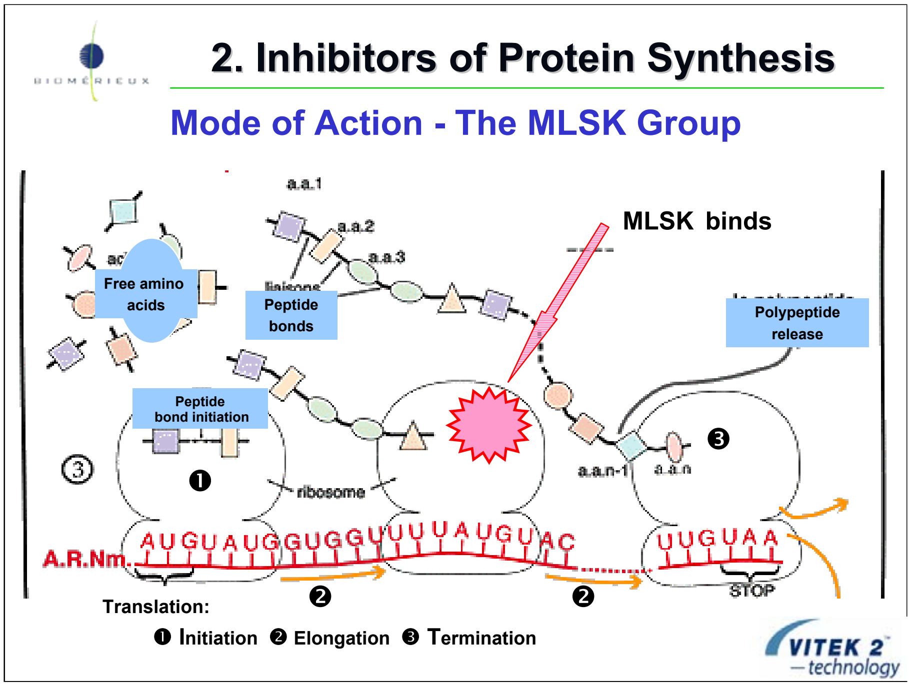


The drugs enter a Gram-positive cell without any problem because peptidoglycan does not act as a barrier for the diffusion of these molecules nor does the cytoplasmic membrane.

Target = Ribosome in cytoplasm



- In Gram-negative bacteria there is no entry because MLSK are lipophilic molecules. They cannot cross the outer membrane which is hydrophilic. “Oil and water don’t mix”.
- MLSK are also large molecules that cannot pass through the porins (which are also aqueous channels) – impermeability.
- **Most Gram-negatives are naturally resistant to MLSK**



- Antibiotics in the MLSK group are structurally distinct but have a similar mode of action by binding the 50S ribosomal subunit
- During translation, it blocks the initiation step, elongation step or peptide release step of protein synthesis
- Unfinished or toxic protein is released

2. Inhibitors of Protein Synthesis

Aminoglycosides

MLSK
(Macrolides, Lincosamides, Streptogramins, Ketolides)

- Tetracyclines
- Glycylcyclines

NEW

Phenicols

Oxazolidinones

Ansamycins

VITEK 2 technology

Inhibitors of Protein Synthesis

Tetracyclines:

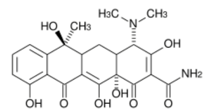
- Bacteriostatic
- Chlortetracycline – 1948, the original tetracycline derived from a *Streptomyces spp*

Glyclycyclines:

- New class
- Developed to overcome some of the more common tetracycline resistance mechanisms
- Bacteriostatic
- Broad spectrum

2. Inhibitors of Protein Synthesis

Tetracyclines
Glycylcyclines

Tetracyclines	International Common Name
	Tetracycline Minocycline Doxycycline
Glycylcyclines	Tigecycline

Inhibitors of Protein Synthesis

Tetracyclines:

Spectrum of Action: Broad spectrum, but resistance is common which limits its use. Primarily for treatment of genital infections (chlamydiae) and atypicals (Rickettsiae, Mycoplasma). Growth promotor in animal husbandry.

Toxicity: Diffuse well in cells and bones. Not recommended for pregnant women and children (less than 2 years old) because of the toxicity on bones and teeth of the fetus.

Tetracycline = Short acting

Minocycline and Doxycycline = Long acting

Minocycline and Doxycycline are more active than Tetracycline.

if Tetra = S, then Mino and Doxy = S

if Tetra = R, must test Mino and Doxy (may be S)

Glycylcyclines: Tigecycline

Spectrum of Action: Same as the tetracyclines; may have activity against multi-drug resistant organisms.

Derivative of Minocycline.

(VT2 - available for Gram Negs.
Must add breakpoints in 2.01 PC software.
Breakpoints are included in 3.01 PC software.
FDA Gram-negative breakpoints = 2/4/8.)

2. Inhibitors of Protein Synthesis

Mode of Action - Tetracycline

	Mode of Action	Effect
Tetracyclines	Irreversibly binds to the 30S ribosomal sub-unit	Inhibits elongation step of protein synthesis

Tetracycline exists as a mixture of two forms - lipophillic and hydrophillic.

- Helps the antibiotic gain entry into the Gram-pos and neg cell
- Once inside the cell it complexes with Mg++ ions, making the molecule bigger and trapping it inside the bacterial cell
- It can then go on to reach it's target - the 30s ribosome
- There it inhibits the elongation step of protein synthesis

Gram-positives have no natural resistance to the tetracyclines.

Of the Gram-negative organisms only Proteus mirabilis is naturally resistant.

2. Inhibitors of Protein Synthesis

Aminoglycosides

MLSK
(Macrolides, Lincosamides, Streptogramins, Ketolides)

Tetracyclines

Glycylcyclines

➢ **Phenicols**

Oxazolidinones

Ansamycins

VITEK 2 technology

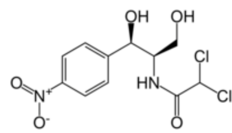
Inhibitors of Protein Synthesis

Phenicols:

- Bacteriostatic
- Broad spectrum

2. Inhibitors of Protein Synthesis

Phenicols



Chloramphenicol

Inhibitors of Protein Synthesis

Phenicols: Chloramphenicol

Spectrum of Action:

Very active against many Gram-positive and Gram-negative bacteria, *Chlamydia, Mycoplasma* and *Rickettsiae*. Restricted use for extra-intestinal severe salmonella infection. Empiric treatment of meningitis, crosses blood/brain barrier well.

Toxicity: High toxicity, causes bone marrow aplasia and other hematological abnormalities.

2. Inhibitors of Protein Synthesis

Chloramphenicol

	Mode of Action	Effect
Chloramphenicol	Binds to the 50S ribosomal sub-unit	Inhibits elongation step of protein synthesis

- Relatively small molecule, easily enters Gram-positive and Gram-negative bacteria
- Target is Ribosome
- Binds to 50S subunit where it inhibits elongation step of protein synthesis

2. Inhibitors of Protein Synthesis

Aminoglycosides

MLSK
(Macrolides, Lincosamides, Streptogramins, Ketolides)

Tetracyclines

Glycylcyclines

Phenicols

➢ **Oxazolidinones**

Ansamycins

Inhibitors of Protein Synthesis

Oxazolidinones:

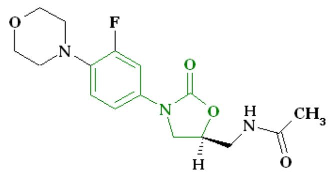
- Bacteriostatic
- Narrow spectrum

2. Inhibitors of Protein Synthesis

Oxazolidinones

NEW

Linezolid



Inhibitors of Protein Synthesis

Oxazolidinones: Linezolid

- Spectrum of Action: Gram-positive infections. Effective for *E. faecium* VRE, MRSA and multi-drug resistant *S. pneumoniae*.
- Trade Name = Zyvox®

2. Inhibitors of Protein Synthesis

Linezolid

	MODE OF ACTION	EFFECT
Linezolid	Binds to the 50S ribosomal sub-unit	Inhibits initiation process of protein synthesis

- Relatively small molecule, easily enters Gram-positive bacteria
- Target is Ribosome
- Linezolid disrupts bacterial growth by inhibiting the initiation process in protein synthesis
- This site of inhibition occurs earlier in the initiation process than other protein synthesis inhibitors (e.g., chloramphenicol, clindamycin, aminoglycosides, and macrolides) that interfere with the elongation process
- Because the site of inhibition is unique to linezolid, cross-resistance to other protein synthesis inhibitors has not yet been reported
- Linezolid may also inhibit virulence factor expression and decrease toxin production in Gram-positive pathogens
- It has been demonstrated that linezolid is bacteriostatic against E*nterococci* and *Staphylococci*, and bactericidal for the majority of *Streptococci*
- Gram-negative bacteria appear to be **naturally resistant**

2. Inhibitors of Protein Synthesis

Aminoglycosides

MLSK

(Macrolides, Lincosamides, Streptogramins, Ketolides)

Tetracyclines

Glycylcyclines

Phenicols

Oxazolidinones

➢ **Ansamycins**

Inhibitors of Protein Synthesis

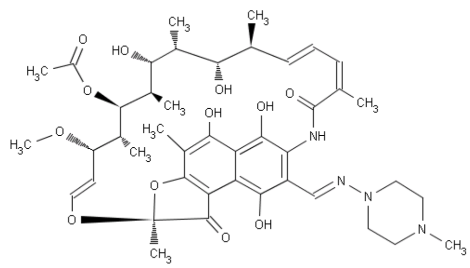
Ansamycins → Rifamycins → Rifampin (Rifamipicin):

- Bacteriostatic or bactericidal – depending on organism and concentration
- Broad spectrum
- Discovered in 1959
- Natural or semi-synthetic derivitive of *Amycolatopsis rifamycinica* (previously *Streptomyces mediterranei*)

2. Inhibitors of Protein Synthesis

Ansamycins

Rifampin (Rifampicin)



VITEK 2 technology

Inhibitors of Protein Synthesis

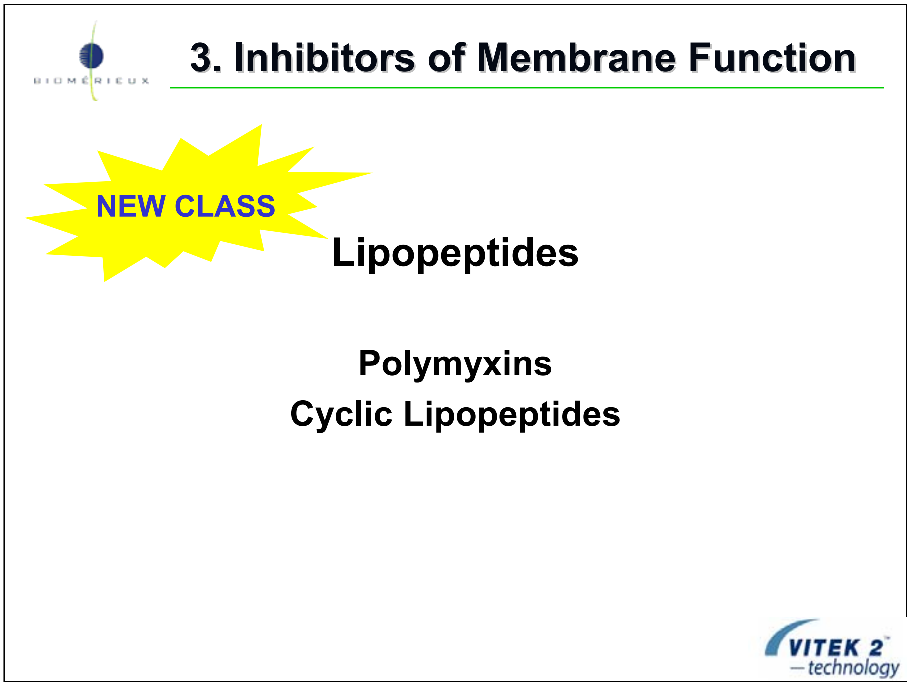
Ansamycins → Rifamycins → Rifampin (Rifamipicin):

Spectrum of Action:

- Primarily Gram-positive organisms and some Gram-negatives
- Used in combinations with other drugs to treat tuberculosis
- Used to treat carriers of *N.meningitidis* (prophylaxsis)
- Used in combination with other antibiotics for severe *Staphycoccal* infections (including MRSA)
- Oral or IV administration

Mode of Action:

Forms a stable complex with RNA polymerase and prevents DNA from being transcribed into RNA, thus inhibiting protein synthesis



Inhibitors of Membrane Function

Lipopeptides: (previously Polypeptides)

Polymyxins have been around since 1940’s and 1950’s.

Polymyxin A,B,C,D,E

- Polymyxin B and E can be used therapeutically
- Polymyxin B – derived from Bacillus polymyxa var. aerosporus
- Polymyxin E – derived from Bacillus polymyxa var. colistinus = Colistin

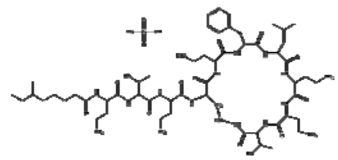
  Colistin exsists as two forms:
  - Colistin sulfate – intestinal infections, topical, powders, media
  - **Colistimethate sodium** – most active, effective form

- All Bactericidal

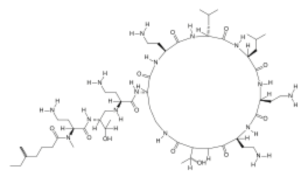
3. Inhibitors of Membrane Function

- **Polymyxins**

Polymyxin B



Colistin



Inhibitors of Membrane Function

Lipopeptides: Polymyxins

Polymyxin B

Spectrum of Action:

Narrow spectrum for Gram-negative UTI, blood, CSF and eye infections. Can be used in combination against very resistant *Pseudomonas*, KPC.

- High toxicity – neurotoxic and nephrotoxic
- IV, IM and topical for infected wounds

Colistin (Colistimethate Sulfate)

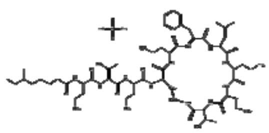
Spectrum of Action:

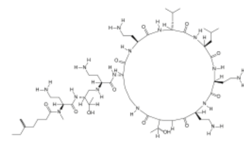
Narrow spectrum for Gram-negatives, especially *Pseudomonas aeruginosa* infections in cystic fibrosis patients. It has come into recent use for treating multi-drug resistant *Acinetobacter* infections. Ineffective for *Proteus* and *Burkholderia.*

- High toxicity – neurotoxic and nephrotoxic
- PO, IV and topical

3. Inhibitors of Membrane Function

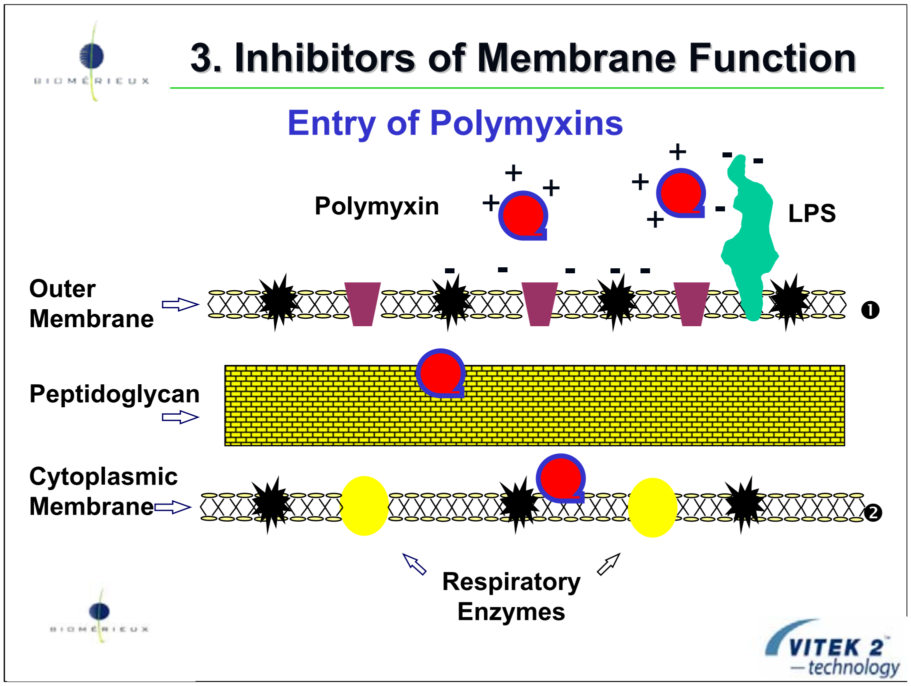
Mode of Action
of
Polymyxins





Lipopeptides

- **Polymyxins**
  - **Polymyxin B**
  - **Colistin**
- Cyclic Lipopeptides
  - Daptomycin



Polymyxin Mode of Action

Target =Membrane phospholipids (lipopolysaccharides (LPS) and lipoproteins)

1. Outer and Cytoplasmic Membrane Effect:

   Polymyxins are positively charged molecules (cationic) which are attracted to the negatively charged bacteria.

   The negative charge of bacteria is due to LPS in the outer membrane and the peptidoglycan (notably the teichoic acid).

   The antibiotic binds to the cell membrane, alters its structure and makes it more permeable. This disrupts osmotic balance causing leakage of cellular molecules, inhibition of respiration and increased water uptake leading to cell death.

   The antibiotic acts much like a cationic detergent and effects all membranes similarly. Toxic side effects are common.

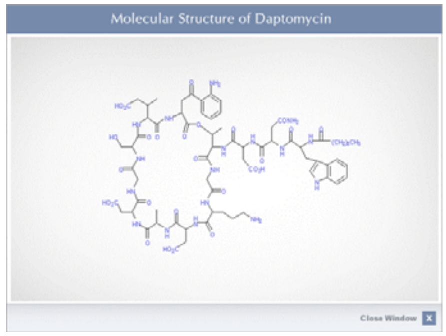
   Little or no effect on Gram-positives since the cell wall is too thick to permit access to the membrane.

   Gram-positives are **naturally resistant.**

3. Inhibitors of Membrane Function

➢ **Cyclic Lipopeptides**

Daptomycin



Inhibitors of Membrane Function

Lipopeptides: Cyclic Lipopeptide

Daptomycin (Cubicin)

- Cubicin from CUBIST Pharmaceutical got FDA approval on September 12, 2003
- Bactericidal
- Resistance rate appears to be low - requires multiple mutations
- Only ‘Sensitive’ CLSI® breakpoints

3. Inhibitors of Membrane Function

Cyclic Lipopeptides

Daptomycin

FDA approval for skin/skin structure infections

- S.aureus (MSSA and MRSA)
- Beta-hemolytic Streptococci (A,B,C,G)
- E.faecalis (Vanco sensitive)

VITEK 2 technology

Inhibitors of Membrane Function

Lipopeptides: Cyclic Lipopeptide

Daptomycin (Cubicin)

Spectrum of Action:

Daptomycin: Daptomycin is bactericidal. Active against Gram-positive bacteria (MRSA, MSSA) including those resistant to methicillin, vancomycin and linezolid.

- 1/day IV dosing
- Requires Ca++ in the media

Mode of Action:

Binds to components (calcium ions) of the cell membrane of susceptible organisms and causes rapid depolarization, inhibiting intracellular synthesis of DNA, RNA, and protein.

Gram-negatives appear to be **naturally resistant**.

4. Anti-Metabolites

(Folate Pathway Inhibitors)

Sulfonamides
Trimethoprim/Sulfamethoxazole

VITEK 2 technology

Anti-Metabolites

- Called folate pathway inhibitors or anti-metabolites

Folic acid is essential for the synthesis of adenine and thymine, two of the four nucleic acids that make up our genes, DNA and chromosomes.

Humans do not synthesize folic acid. Good selective target.

Sulfonamides

- Bacteriostatic
- Introduced in 1930's – first effective systemic antimicrobial agent
- Used for treatment of acute, uncomplicated UTI's

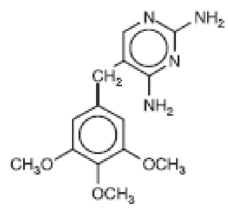
Trimethoprim/Sulfamethoxazole

- TMP/SXT is bactericidal
- Broad spectrum
- Synergistic action

4. Anti-Metabolites

(Folate Pathway Inhibitors)

Trimethoprim/Sulfamethoxazole



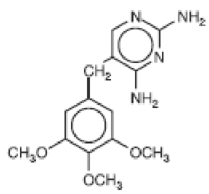
Anti-Metabolites

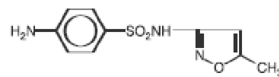
Trimethoprim/Sulfamethoxazole:

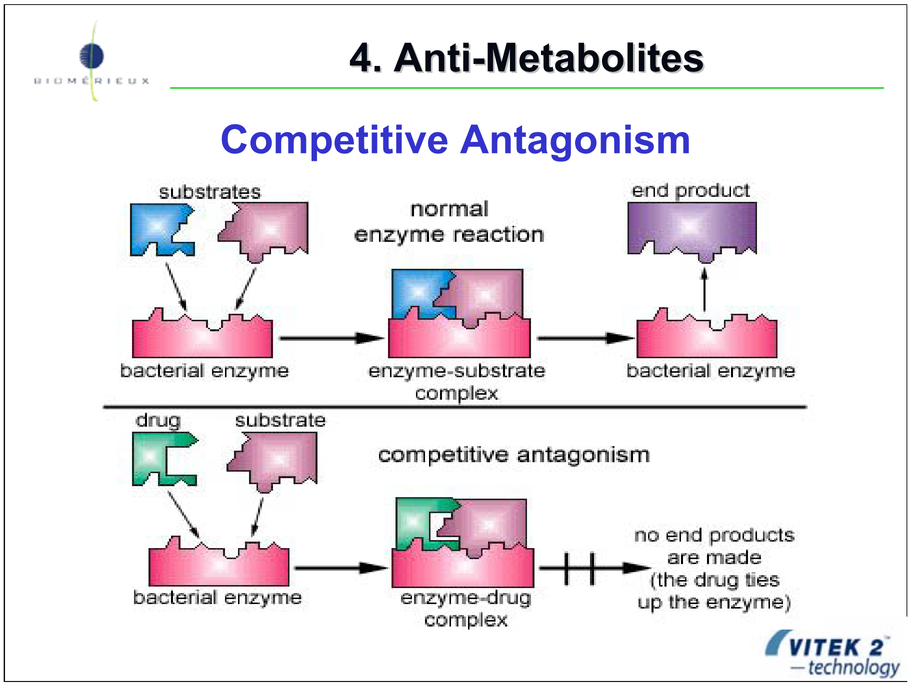
Spectrum of Action: Prescribed for treatment of certain UTI's, otitis media in children, chronic bronchitis in adults, enteritis and Travelers' Diarrhea.

4. Anti-Metabolites

Mode of Action
of
Anti-metabolites

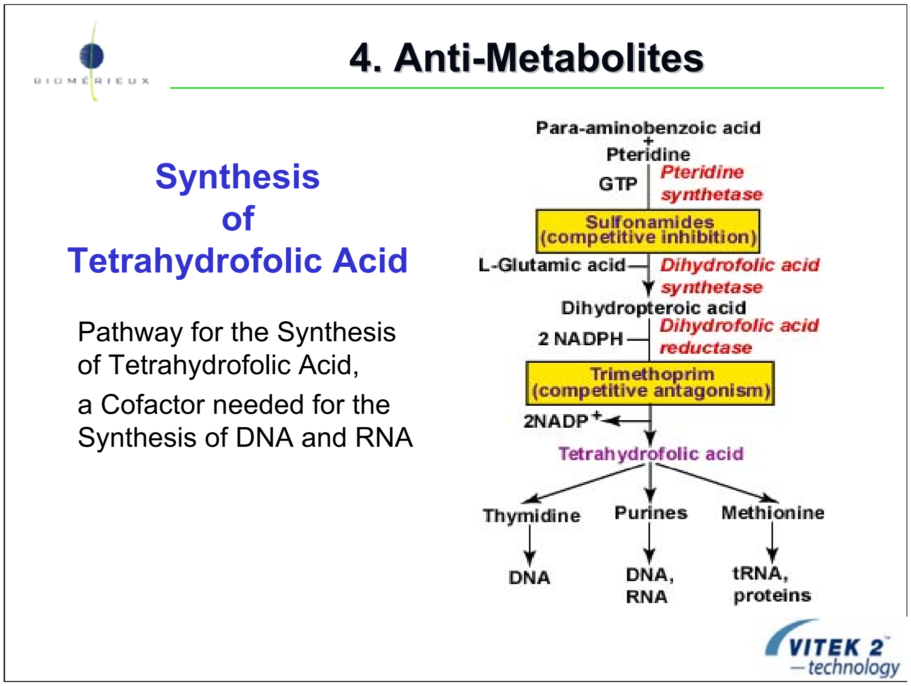






TMP/SXT Mode of Action

- The drug resembles a microbial substrate and competes with that substrate for the limited microbial enzyme
- The drug ties up the enzyme and blocks a step in metabolism



- Sulfonamides such as sulfamethoxazole tie up the enzyme **pteridine synthetase** while trimethoprim ties up the enzyme **dihydrofolic acid reductase**. As a result, tetrahydrofolic acid is not produced by the bacterium.

- Sulfonamides, trimethoprim alone and in combination block folic acid essential for the synthesis of adenine and thymidine that make up the DNA, RNA. Therefore, folate pathway inhibitors do not have direct antibiotic activity but the end result is the same, the bacteria is unable to multiply.

- **The combination SXT (thrimethoprim-sulfamethoxazol) is synergistic and the association provides a bactericidal effect**
- **Natural Resistance**

  Enterococcus – low level and poorly expressed

  S. pneumoniae

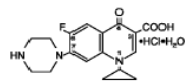
  Ps. aeruginosa (impermeability)

5. Inhibitors of Nucleic Acid Synthesis

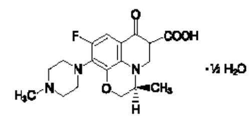
- **Quinolones**
  Furanes

5. Inhibitors of Nucleic Acid Synthesis

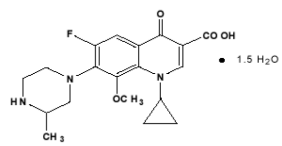
Quinolones



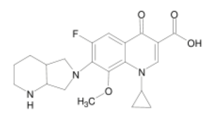
Ciprofloxacin



Levofloxacin



Gatifloxacin



Moxifloxacin

VITEK 2 technology

Inhibitors of Nucleic Acid Synthesis

Quinolones:

- Humans do synthesize DNA - shared process with bacteria
- Do tend to see some side effects with Quinolones
- Some drugs withdrawn from market quickly
- All are bactericidal

5. Inhibitors of Nucleic Acid Synthesis

Quinolones

	International Common Name
Quinolones 1st Generation – Narrow Spectrum	Nalidixic Acid Cinoxacin
Fluoroquinolones	Ciprofloxacin Enoxacin Garenoxacin Levofloxacin Lomefloxacin Norfloxacin Ofloxacin Sparfloxacin Gatifloxacin Moxifloxacin Trovafloxacin

VITEK 2 technology

Inhibitors of Nucleic Acid Synthesis

Quinolones:

- **1st Generation Quinolones:** Only for Gram-negatives, used to treat urinary tract infections because they reach high concentrations in the site of infection.
- **Fluoroquinolones: Garenoxacin**

  Gram-negative and Gram-positive coverage including Anaerobes, Atypicals, *S.pneumoniae* and *Pseudomonas.*

  In development for VT2 cards, not yet approved.
- **Fluoroquinolones: Ciprofloxacin, Levofloxacin, Norfloxacin, Ofloxacin)**

  More effective (lower MIC values).

  Spectrum extended to cover *Staphylococci, Streptococci* and *Pneumococci* (sparfloxacin).

  More widespread tissue distribution (they reach the intestine and the lungs).

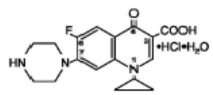
  Ciprofloxacin and Ofloxacin used for systemic infections.
- **Fluoroquinolones: Sparfloxacin, Gatifloxacin, Moxifloxacin**

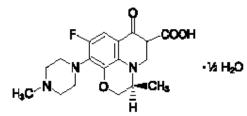
  Trovafloxacin removed from market very quickly after release cardiac arrhythmias, liver destruction, phototoxicity.

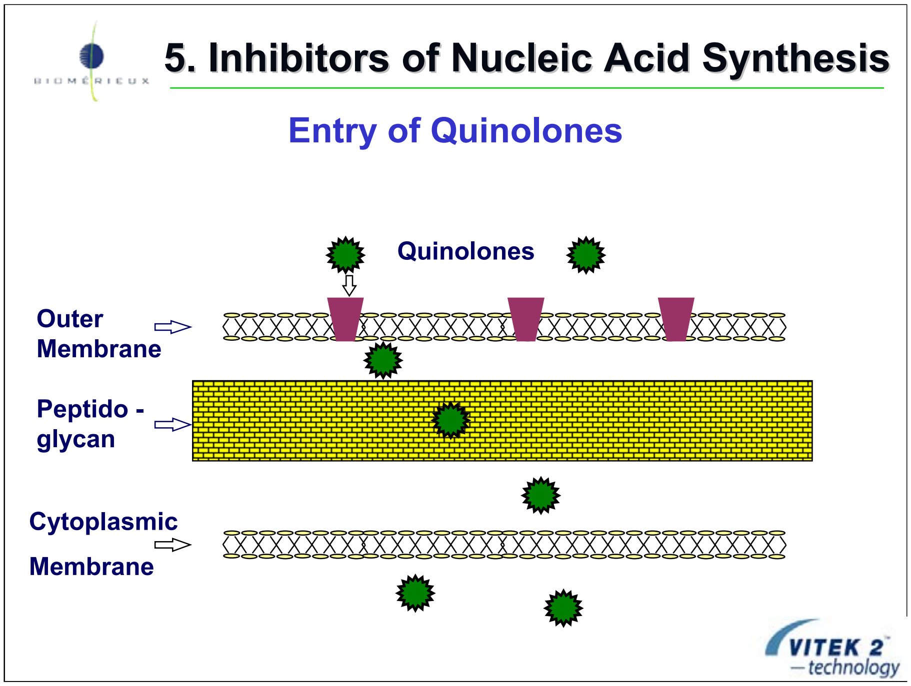
  Gatifloxacin (Tequin®) removed from market 05/01/06 - diabetes.

5. Inhibitors of Nucleic Acid Synthesis

Mode of Action
of
Quinolones

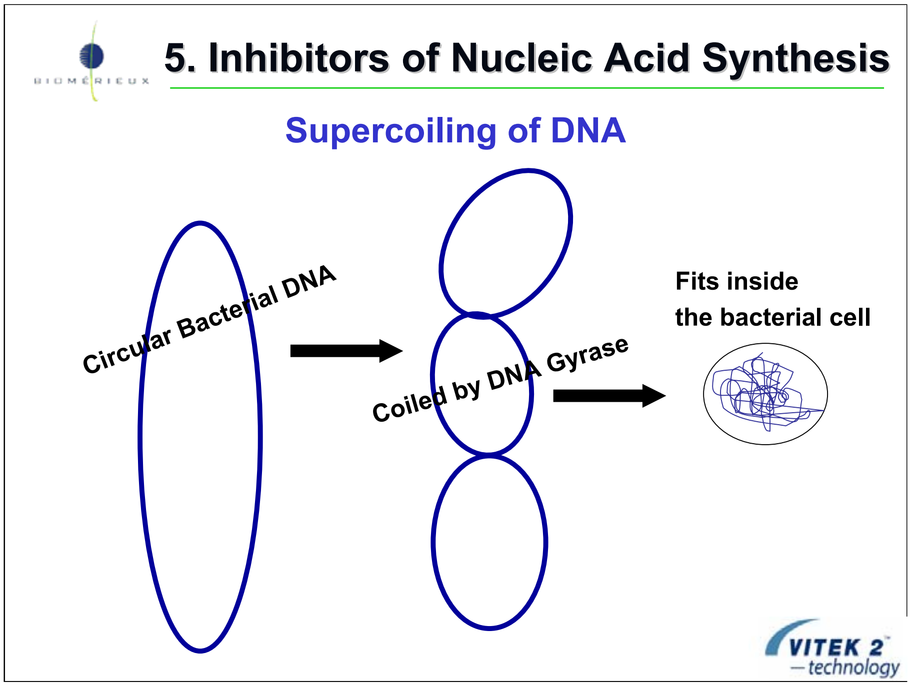




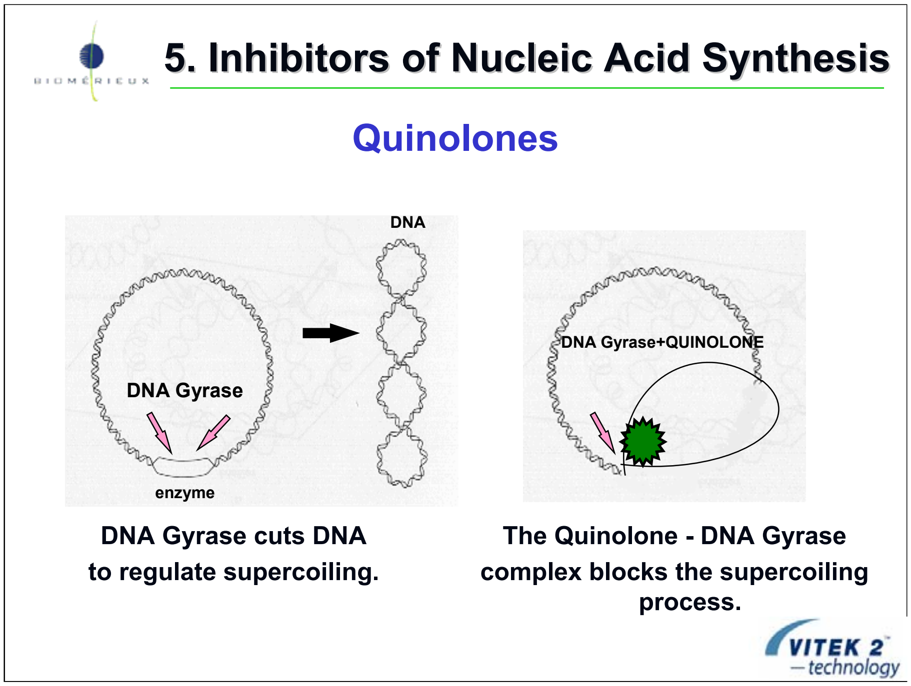


Quinolone Mode of Action

- Small and hydrophilic, quinolones have no problem crossing the outer membrane.
- They easily diffuse through the peptidoglycan and the cytoplasmic membrane and rapidly reach their target.
- Target = Topoisomerases, e.g., DNA-gyrase
- Rapid bactericidal activity



- The bacterial chromosome is supercoiled by the enzyme DNA gyrase
- A fully supercoiled chromosome will be about 1 micron in diameter - small enough to fit in the bacteria
- The bacterial chromosome consists of a single circle of DNA
- DNA is double-stranded forming a left-handed double helix
- A typical *E. coli's* chromosome is 1400 microns long
- The *E. coli's* bacterial cells are 2-3 microns long



- Quinolones inhibit DNA synthesis

- All topoisomerases can relax DNA but only gyrase can also carry out DNA supercoiling (‘supercoiling’ process is necessary to compact the bacterial chromosome which is 1000 times longer than the bacterial cell). Topoisomerases are also involved in DNA replication, transcription and recombination.

- The main quinolone target is the DNA gyrase which is responsible for cutting one of the chromosomal DNA strands at the beginning of the supercoiling process. The nick is only introduced temporarily and later the two ends are joined back together (i.e., repaired).

- The quinolone molecule forms a stable complex with DNA gyrase thereby inhibiting its activity and preventing the repair of DNA cuts

- **Natural Resistance**

  Gram Positives – 1st generation quinolones

  S.pneumoniae – decreased activity to Ofloxacin and Ciprofloxacin

  Ps. aeruginosa – decreased activity to Norfloxacin and Ofloxacin

5. Inhibitors of Nucleic Acid Synthesis

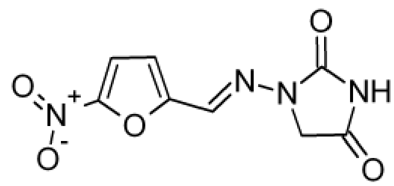
Quinolones

- **Furanes**

5. Inhibitors of Nucleic Acid Synthesis

Furanes

Nitrofurantoin



Nitrofurans: Nitrofurantoin

- Spectrum of Action: Urinary Tract Infections caused by Gram-negative and Gram-positive organisms
- Broad spectrum
- Bactericidal
- Oral

Mode of Action:

The drug works by damaging bacterial DNA. In the bacterial cell, nitrofurantoin is reduced by flavoproteins (nitrofuran reductase). These reduced products are are highly active and attack ribosomal proteins, DNA, respiration, pyruvate metabolism and other macromolecules within the cell. It is not known which of the actions of nitrofurantoin is primarily responsible for its bactericidal acitivity.

Natural Resistance:

Pseudomonas and most *Proteus spp*. are naturally resistant.

Antibiotics and Modes of Action

Questions?