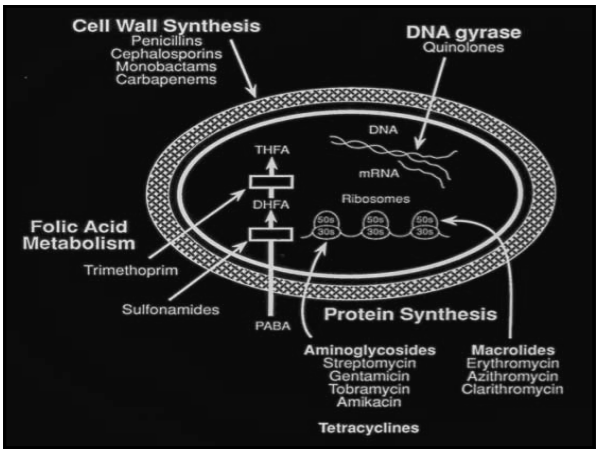


Bacterial modifications:

1 – Mutate target - ? More than one protein
Importance of the target –
? Essential

2. Permeability – Size/charge considerations
? Substrate for an efflux pump

3. Selection for mutants that destroy the antibiotic

Beta-lactam antibiotics

Penicillins

Target - Cell wall - interfere with cross linking
Actively growing cells

Bind to **Penicillin Binding Proteins**

Enzymes involved in cell wall synthesis

WHO discovered the penicillins??

Abess Hildegarde von Bingen ?

"Good things that grow on the sides of trees…."

Fleming –

Florey – WWII….

Activity of an Antibiotic

Affinity for target

Permeability properties
(ability to get to the target)

Stability to bacterial enzymatic degradation

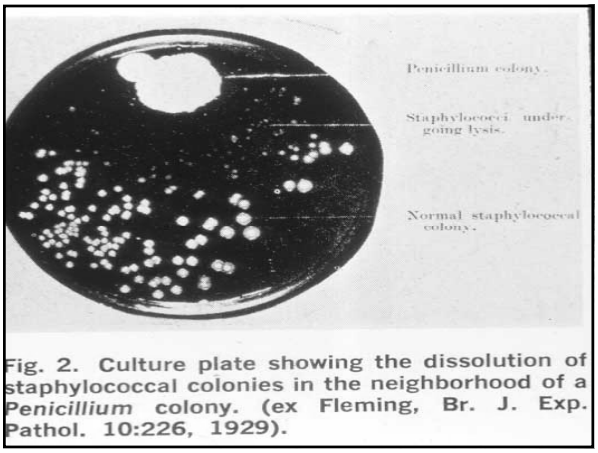
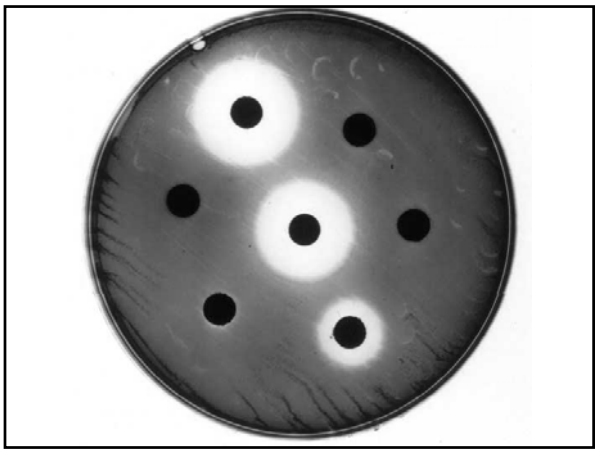


Fig. 2. Culture plate showing the dissolution of staphylococcal colonies in the neighborhood of a *Penicillium* colony. (ex Fleming, Br. J. Exp. Pathol. 10:226, 1929).



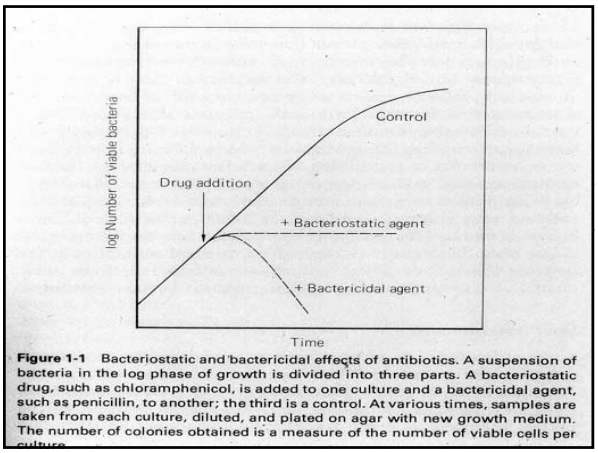
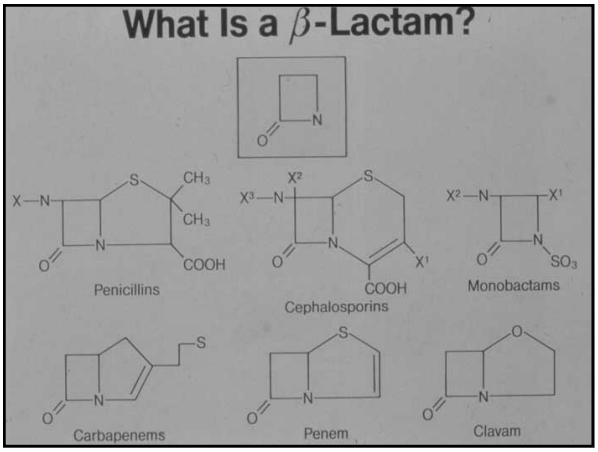
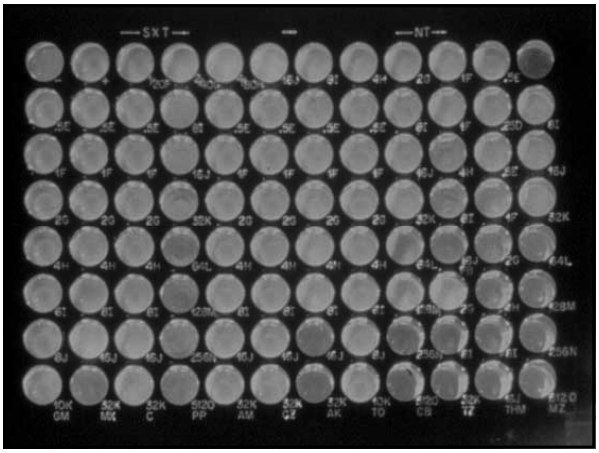
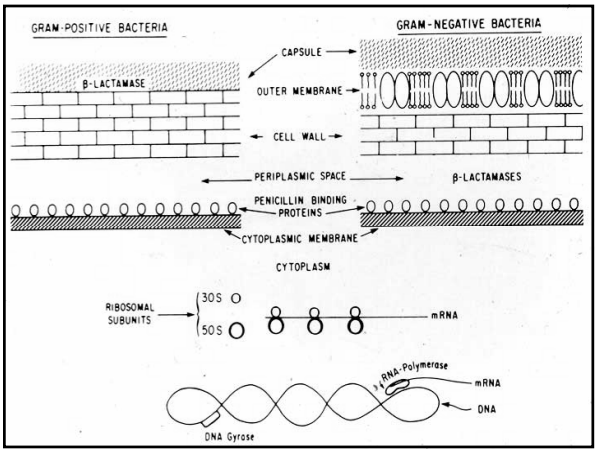
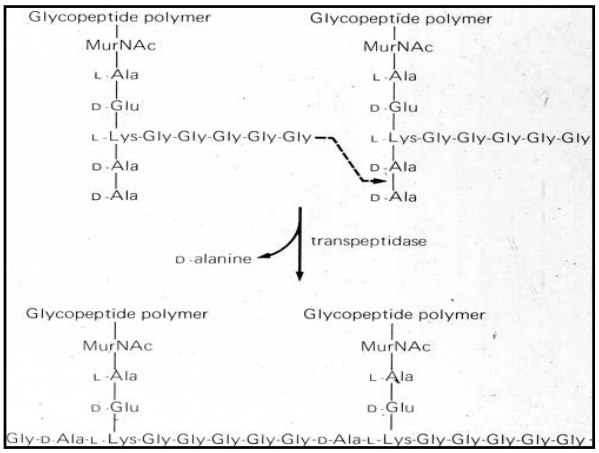


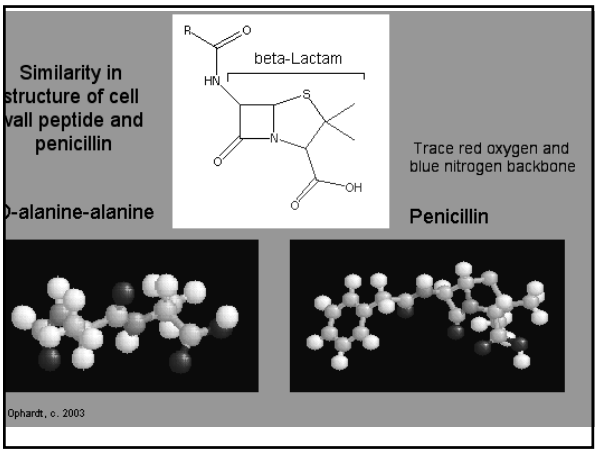
Figure 1-1 Bacteriostatic and bactericidal effects of antibiotics. A suspension of bacteria in the log phase of growth is divided into three parts. A bacteriostatic drug, such as chloramphenicol, is added to one culture and a bactericidal agent, such as penicillin, to another; the third is a control. At various times, samples are taken from each culture, diluted, and plated on agar with new growth medium. The number of colonies obtained is a measure of the number of viable cells per culture.











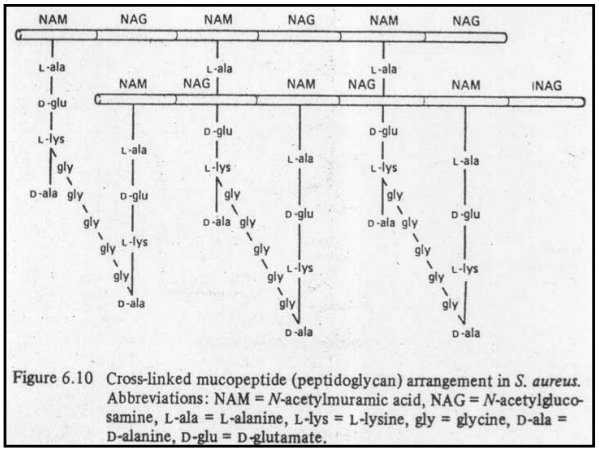


Figure 6.10 Cross-linked mucopeptide (peptidoglycan) arrangement in *S. aureus*. Abbreviations: NAM = *N*-acetylmuramic acid, NAG = *N*-acetylglucosamine, L-ala = L-alanine, L-lys = L-lysine, gly = glycine, D-ala = D-alanine, D-glu = D-glutamate.

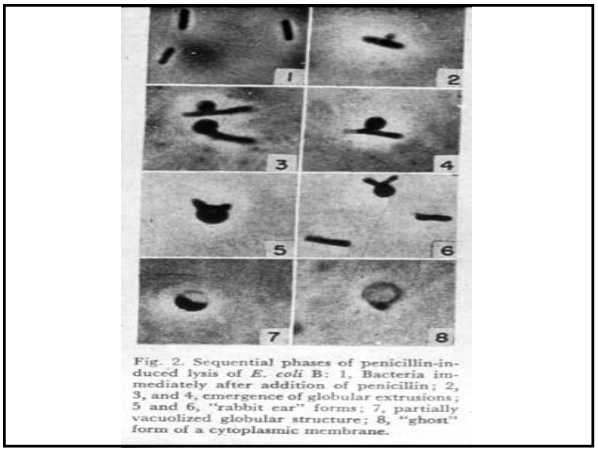


Fig. 2. Sequential phases of penicillin-induced lysis of *E. coli* B: 1, Bacteria immediately after addition of penicillin; 2, 3, and 4, emergence of globular extrusions; 5 and 6, "rabbit ear" forms; 7, partially vacuolized globular structure; 8, "ghost" form of a cytoplasmic membrane.

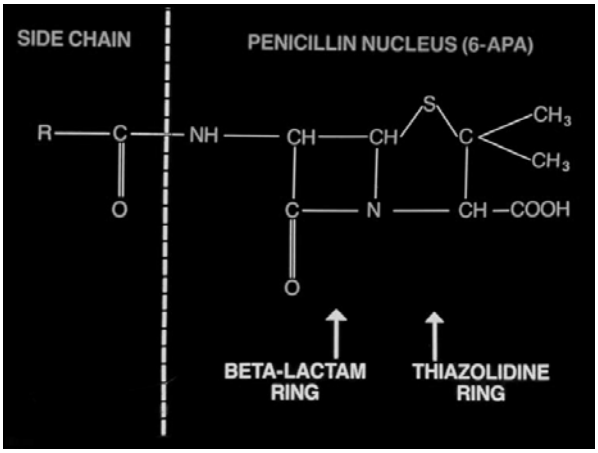
Penicillin binding proteins

Transpeptidases

Carboxypeptidases

Differ in Gram (+) and in Gram (-) bacteria

Differ in abundance



Activity of the beta-lactam antibiotic:

Affinity for critical PBP's (number of copies of the target)

Ability to get to the target (permeability properties – more of an issue for Gram negs)

Stability to beta-lactamases - degradation

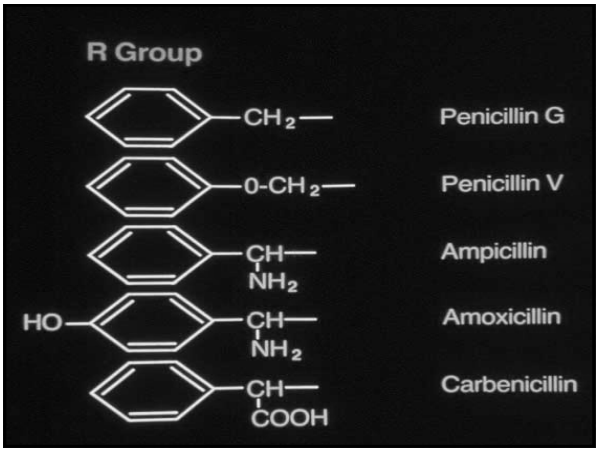
ANTI-staphylococcal penicillins

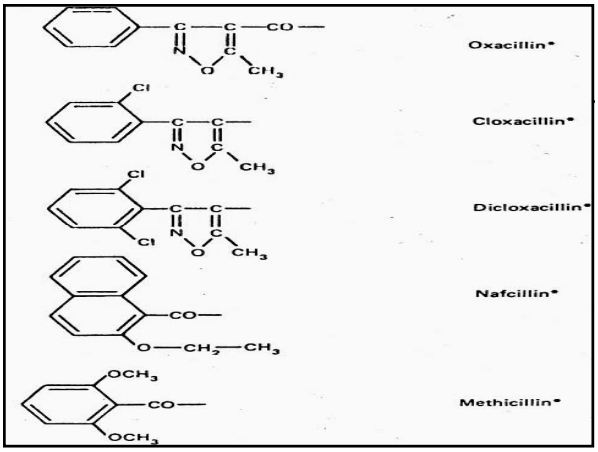
"semi-synthetic"

Add bulky side chains to provide

STERIC HINDRANCE to protect the Beta-lactam nucleus –

Gram positives – secrete bla's – "cloud"





Beta-lactamases - cleave the beta-lactam ring - inactivate the drug -
Open ring - can't bind to the target

Co-evolved with the penicillin binding proteins

Share a ser-X-X-lys - binding site for interactions

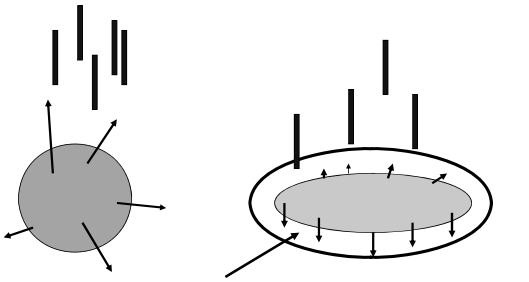
Gram positives - Secreted into the environment
Gram negatives - Secreted into the periplasmic space

Anti-staphylococcal penicillins

Strategy - Add a bulky side group to block beta-lactamase

(Methicillin) - renal toxicity
Nafcillin
Oxacillin
Cloxacillin (di-clox) - oral drugs

Beta-lactamase secretion in Gram positive and Gram negative bacteria



Periplasmic Space – Gram Negative

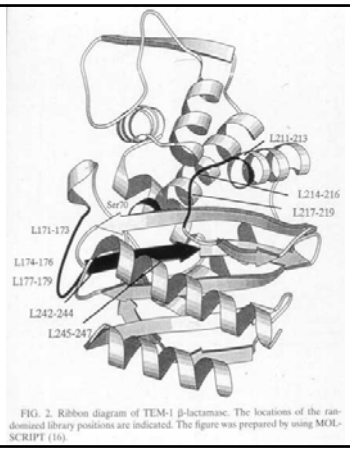
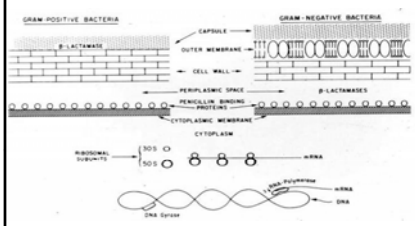


FIG. 2. Ribbon diagram of TEM-1 β-lactamase. The locations of the randomized library positions are indicated. The figure was prepared by using MOLSCRIPT (16).

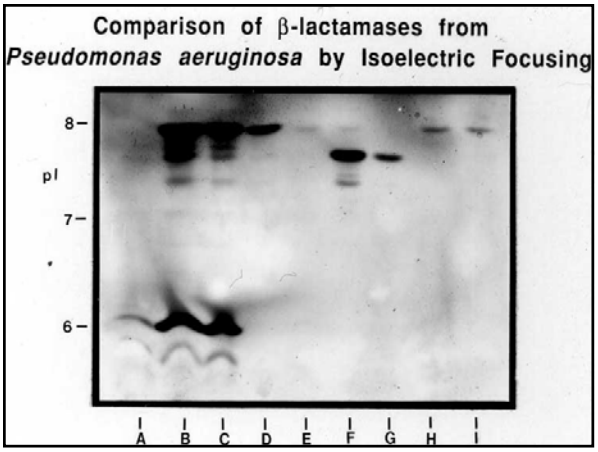


Capsule – No barrier

Peptidoglycan – No barrier

Outer wall – Porins Control access

Cytoplasmic Membrane – Lipid Bilayer



Beta-lactamases

Regulation - Consititutive - Chromosomal (*E*.coli)

Plasmid mediated - copy number dependent

Inducible - chromosomal - SPACE organisms - as a model

2-component signaling - (*ampD, ampE, ampR*)
- Sensor
- Response regulator
- Transcriptional activator

Drugs in clinical use:

Penicillin G, VK

Ampicillin (+) clavulanic acid (beta-lactamase inhibitor)

(oral or parenteral)

Piperacillin - anti-Pseudomonas (+tazobactam) (parenteral)

Spectrum - gram positive and gram negative -
- Not inherently beta-lactamase stable
- Spectrum - dependent upon permeability properties

Add a beta-lactamase inhibitor

Clavulanic acid -
Sulbactam
Tazobactam

Expands spectrum of activity
Anaerobes

NOT effective against the beta-lactamases of the SPACE organisms

Pharmacology of the penicillins

Absorption - Amoxicillin - acid stable
dosing - give more - longer intervals
Augmentin - amox + clav - diarrhea

Metabolism - minor

Excretion - Renal - tubular secretion
Increase serum levels with probenecid
Biliary - only ureido penicillins
Nafcillin

Distribution - Anions - charged - extracellular space
CSF - with inflammation
Concentrated in urine