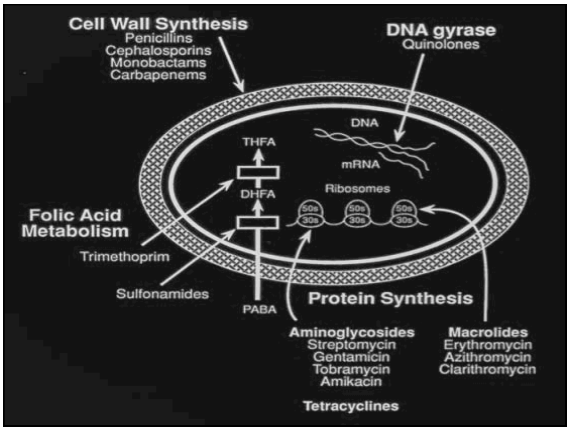


Beta-lactam antibiotics

Penicillins

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

Bind to **Penicillin Binding Proteins**

Enzymes involved in cell wall synthesis

Activity of an Antibiotic

Affinity for target

Permeability properties
(ability to get to the target)

Stability to bacterial enzymatic degradation

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

WHO discovered the penicillins??

Abess Hildegarde von Bingen ?

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

Fleming –

Florey – WWII….

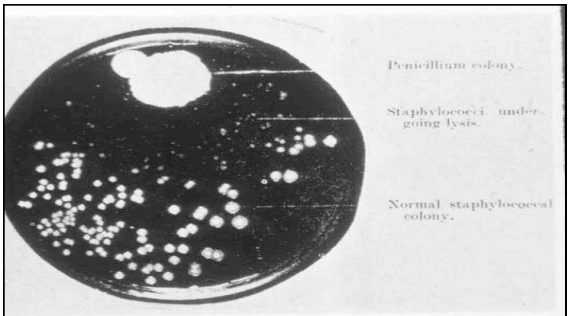
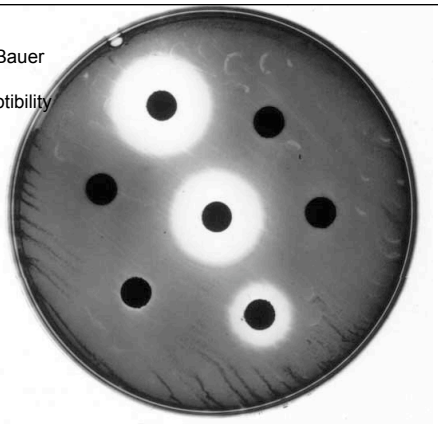
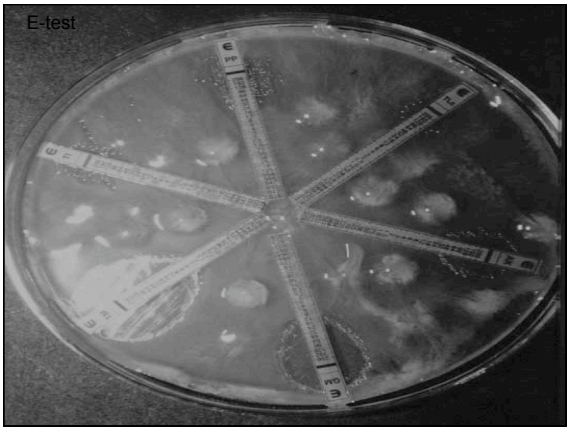


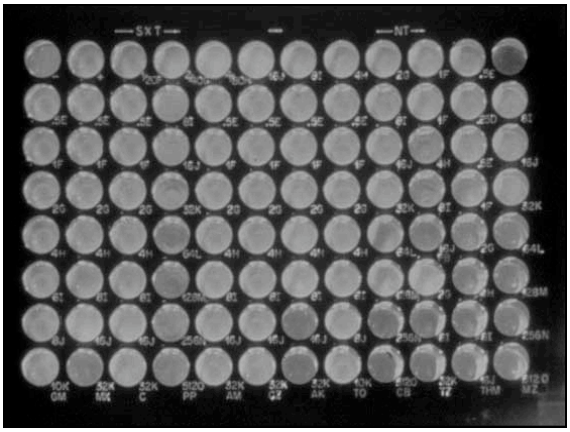
Fig. 2. Culture plate showing the dissolution of staphylococcal colonies in the neighborhood of a *Penicillium* colony. (ex Fleming, Br. J. Exp. Pathol. 10:226, 1929).

Kirby-Bauer
Disc
susceptibility



E-test





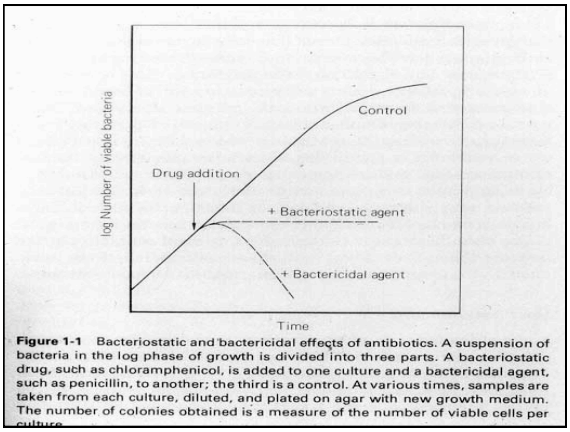
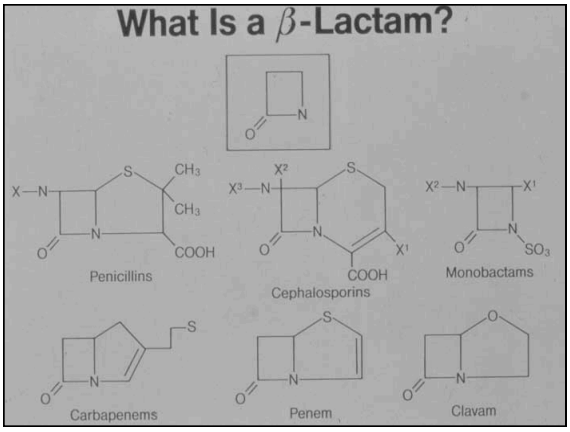
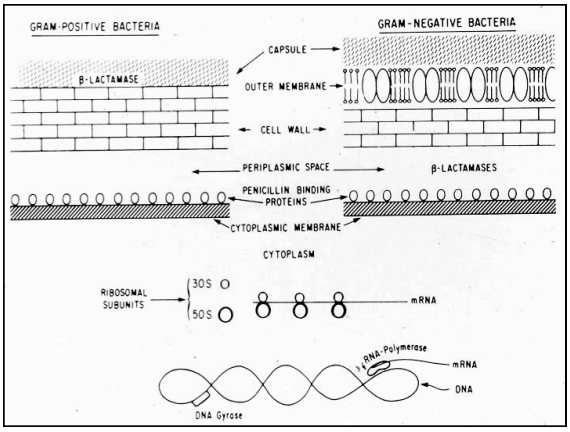
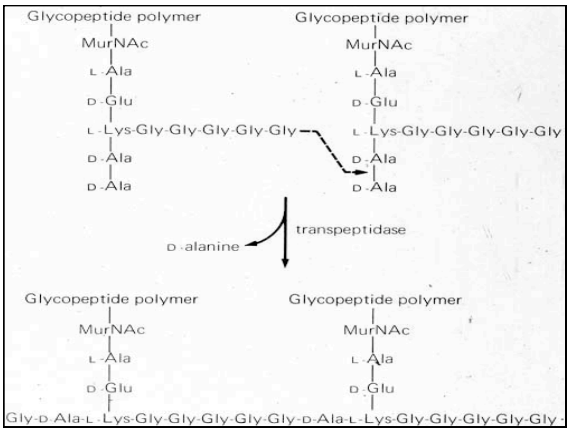


Figure 1-1 Bacteriostatic and bacteriocidal effects of antibiotics. A suspension of bacteria in the log phase of growth is divided into three parts. A bacteriostatic drug, such as chloramphenicol, is added to one culture and a bacteriocidal agent, such as penicillin, to another; the third is a control. 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.







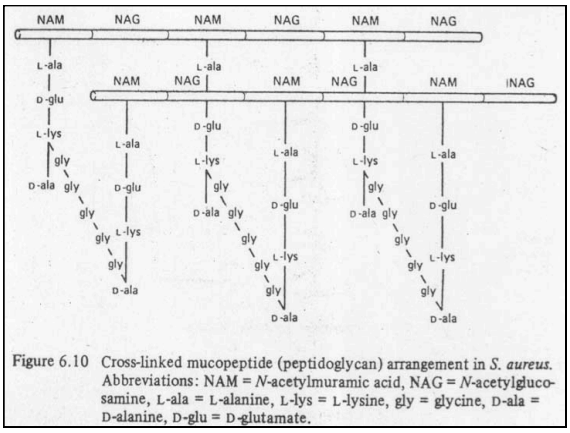


Figure 6.10 Cross-linked mucopeptide (peptidoglycan) arrangement in *S. aureus*. Abbreviations: NAM = *N*-acetylmuramic acid, NAG = *N*-acetylglucosamine, L-ala = L-alanine, L-lys = L-lysine, gly = glycine, D-ala = D-alanine, D-glu = D-glutamate.

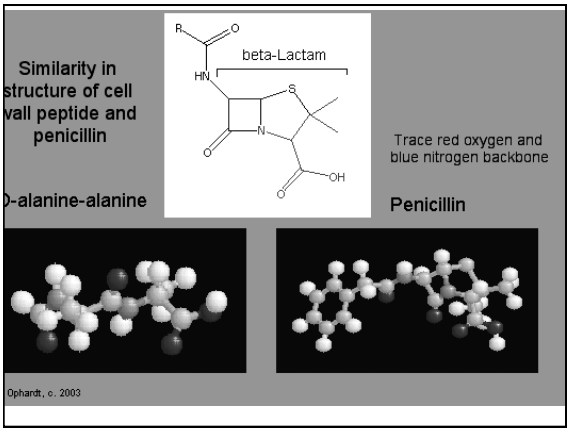
Penicillin binding proteins

Transpeptidases

Carboxypeptidases

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

Differ in abundance



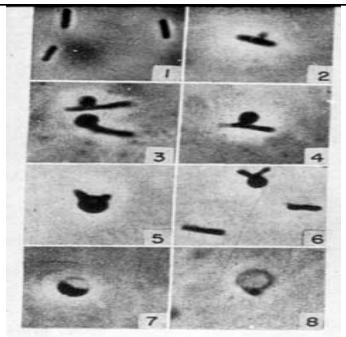
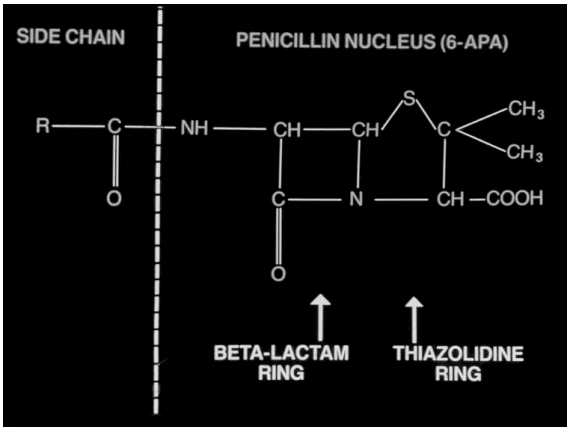


Fig. 2. Sequential phases of penicillin-induced lysis of *E. coli* B: 1, Bacteria immediately after addition of penicillin; 2, 3, and 4, emergence of globular extrusions; 5 and 6, "rabbit ear" forms; 7, partially vacuolized globular structure; 8, "ghost" form of a cytoplasmic membrane.

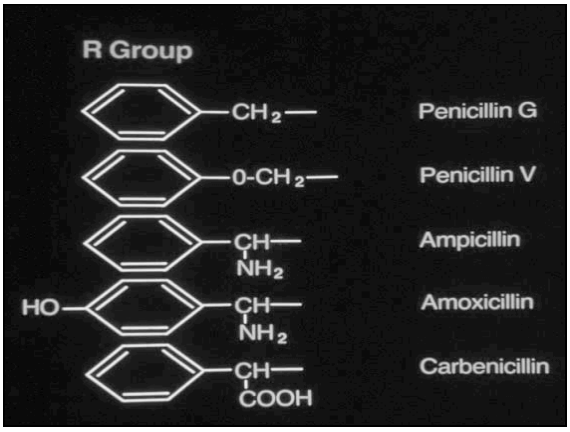


Activity of the beta-lactam antibiotic:

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



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

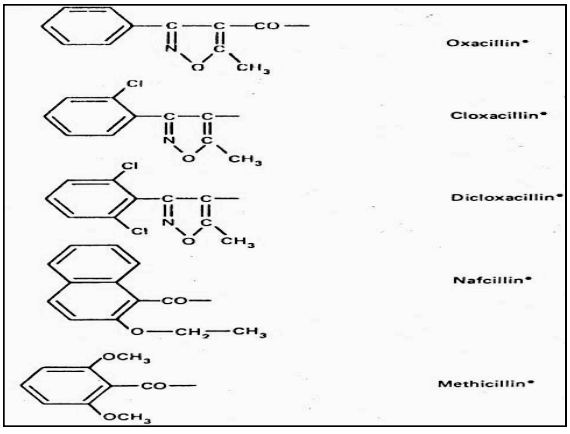
ANTI-staphylococcal penicillins

"semi-synthetic"

Add bulky side chains to provide

STERIC HINDRANCE to protect the Beta-lactam nucleus –

Gram positives – secrete bla's – "cloud"

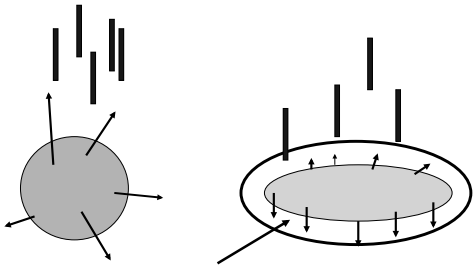


Anti-staphylococcal penicillins

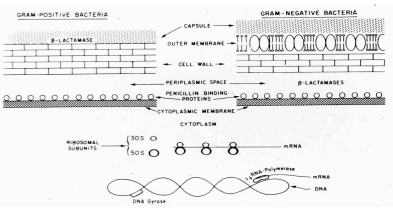
Strategy - Add a bulky side group to block beta-lactamase

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

Beta-lactamase secretion in Gram positive and Gram negative bacteria



Periplasmic Space – Gram Negative



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

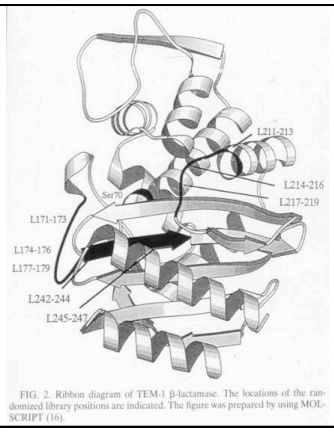
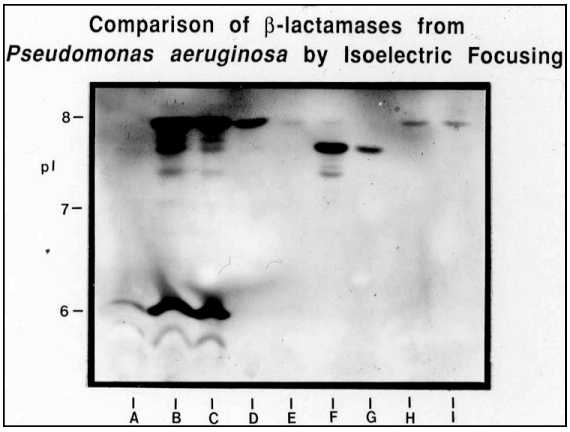


FIG. 2. Ribbon diagram of TEM-1 β-lactamase. The locations of the randomized library positions are indicated. The figure was prepared by using MOLSCRIPT (16).



Drugs in clinical use:

Penicillin G, VK

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

(oral or parenteral)

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

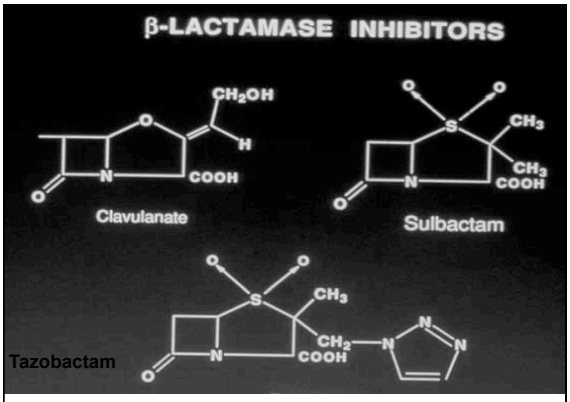
Spectrum - gram positive and gram negative -
- Not inherently beta-lactamase stable
- Spectrum - dependent upon permeability properties

Add a beta-lactamase inhibitor

Clavulanic acid -
Sulbactam
Tazobactam

Expands spectrum of activity
Anaerobes

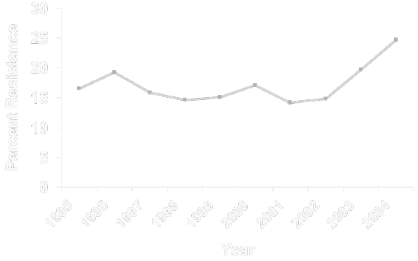
NOT effective against the beta-lactamases of the SPACE organisms



Multiple mechanisms of resistance

- Porins - limit access to PBPs
- Efflux pumps - pump out drugs
- Inducible chromosomal beta-lactamases
- Constitutive plasmid mediated beta-lactamases
- Altered PBPs

3rd generation cephalosporin-resistant *Kliebsiella pneumoniae* Among ICU Patients, 1995-2004



Source: National Nosocomial Infections Surveillance (NNIS) System

Rapid Spread of Carbapenem-Resistant Klebsiella pneumoniae in New York City

A New Threat to Our Antibiotic Armamentarium

Simona Bratu, MD; David Landman, MD; Robin Haag, RN; Rose Recco, MD; Antonella Eramo, RN; Maqsood Al

Arch Intern Med. 2005;165:1430-1435.

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 2006, p. 3396–3406 Vol. 50, No. 10 0066-4804/06/$08.000.

High-Level Carbapenem Resistance in a Klebsiella pneumoniae Clinical Isolate Is Due to the Combination of blaACT-1 -Lactamase Production, Porin OmpK35/36 Insertional Inactivation, and Down-Regulation of the Phosphate Transport Porin PhoE

Frank M. Kaczmarek, Fadia Dib-Hajj, Wenchi Shang, and Thomas D. Gootz* Pfizer Global Research and Development, Groton, Connecticut 06340 7 July 2006

ESBL risk factors

Extended spectrum beta-lactamase production

Hospital associated infections
Indwelling catheter
Mechanical ventilation
Increased length of stay
ICU stays
Gut colonization
Emergency surgery

NEJM Vol 352:380-391 2005

The New {beta}-Lactamases
George A. Jacoby, M.D., and Luisa Silvia Munoz-Price, M.D.

Development of resistance:

1 - Acquisition of genetic material - transposons, IS elements
plasmids

2- Selection of porin mutations - altered uptake

3- Induction of efflux pumps -

Multiple genetic mechanisms

Penicillin resistant *S. pneumoniae*

- Strain 19A - Otitis media (community)
- Altered PBPs
- PRSP (pen resistant versus PISP (intermediate susceptibility)
- NOT susceptible to FDA approved oral drugs
- ? Fluoroquinolone susceptible

Cases of Pneumococcal Acute Otitis Media by Serotype and Respiratory Season Among Rochester, New York, Children JAMA, Oct 2007

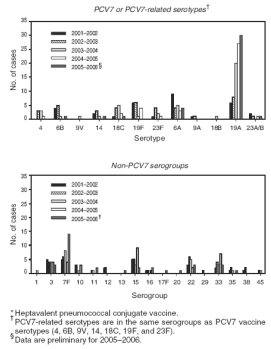
Year

No. of Cases

PCV7 Serotype 4 6B 9V 14 18C 19F	**Non-PCV7 Serotype** 3 6A 9V 11 14 15 19A 22F 23A 33F 35B
2003-2004 1 4 1 1 9 16 (57 %)	3 1 2 2 2 1 1 12 (43%)
2004-2005 1 1 2 4 (33)	1 2 1 2 1 1 8 (66 %)
2005-2006 1 4 5 (26)	3 1 1 5 1 1 1 1 14 (74%)

Abbreviation: PCV7, pneumococcal conjugate vaccine containing 7 serotypes.
aThe **decrease** in isolation of PCV7 serotypes and **increase** in non-PCV7 serotypes over time are both significant at P .001.
Total number of *Streptococcus pneumoniae* isolates for each respiratory season and the percentage for that season.

FIGURE 2. Number of cases of invasive pneumococcal disease among persons aged <18 years, by PCV7* status of *Streptococcus pneumoniae* serotypes — Massachusetts, October 1, 2001–September 30, 2006



* Heptavalent pneumococcal conjugate vaccine.
† PCV7-related serotypes are in the same serogroups as PCV7 vaccine serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F).
§ Data are preliminary for 2005–2006.

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