I. Antibiotics: Overview

A. Definitions

Definition: Antibiotics are molecules that kill, or stop the growth of, microorganisms, including both bacteria and fungi.

Antibiotics that kill bacteria are called "bactericidal"
Antibiotics that stop the growth of bacteria are called "bacteriostatic"

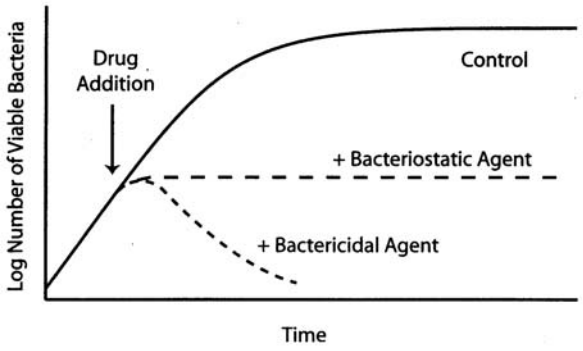
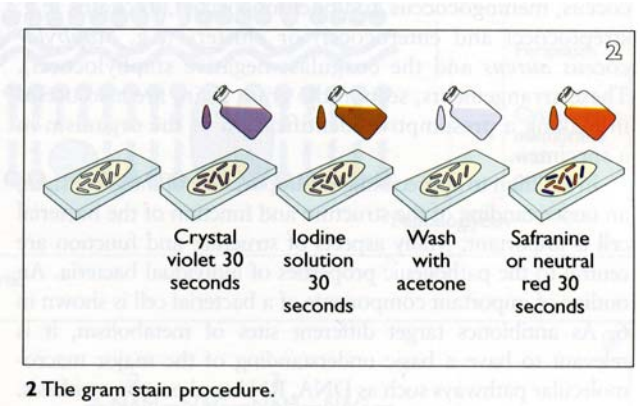


Figure 1.1 Effects of bacteriostatic versus bactericidal antibiotics on a logarithmically growing bacterial culture. (From Scholar and Pratt [2000], with permission.)

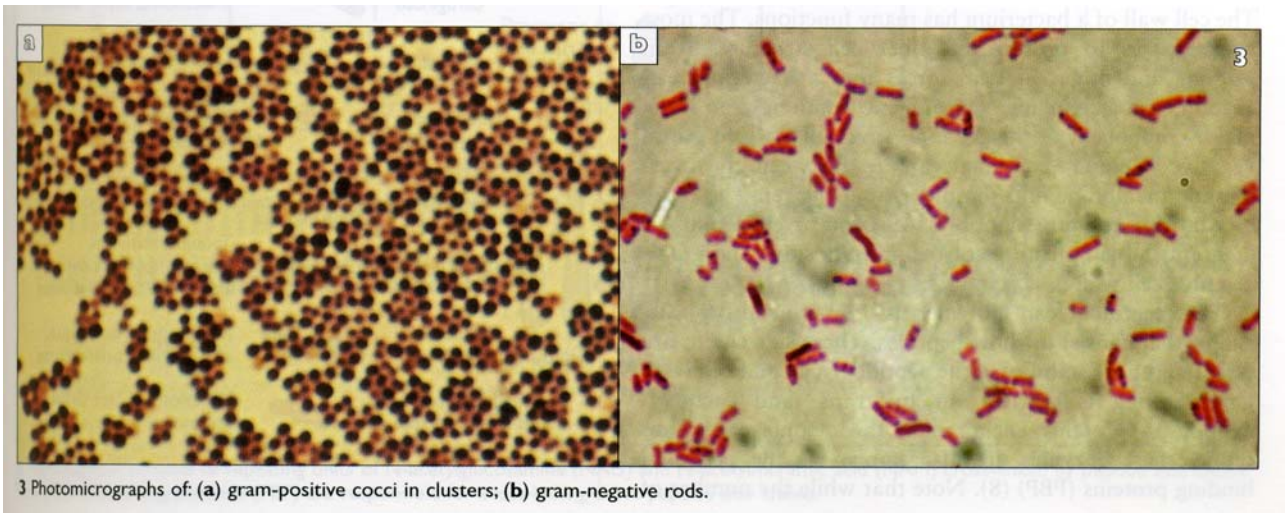
B. Why do we want to kill bacteria?

Types of bacteria: Gram stain

- A test, resulting in the classification of bacteria, developed in the last century by Hans Christian Gram, a Danish microbiologist
- Gram positive bacteria will retain the original blue stain
- Gram negative bacteria will lose the blue stain upon intermediate acetone treatment and will stain red



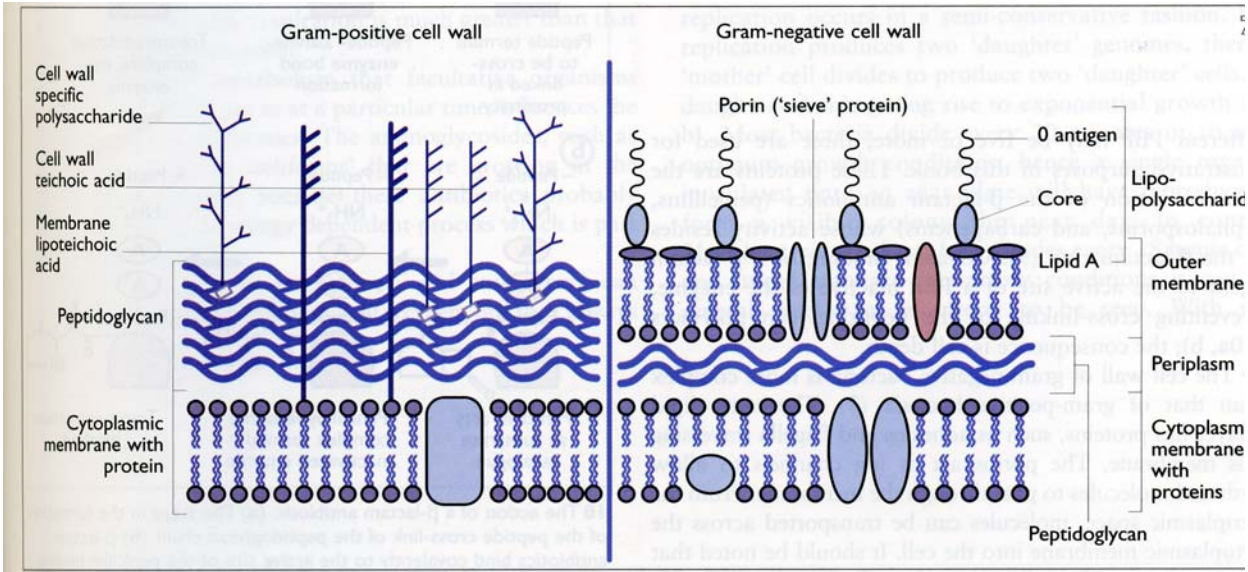
2 The gram stain procedure.



3 Photomicrographs of: (**a**) gram-positive cocci in clusters; (**b**) gram-negative rods.

5

Organism	Gram stain features	Clinical importance – some examples
Aerobic/facultative bacteria		
Enterococci		Urinary tract infections, endocarditis
Streptococci A,B,C,D,G		A: pharyngitis, cellulitis B: neonatal sepsis
Viridans streptococci		Endocarditis, abscess, dental caries
Streptococcus pneumoniae		Community pneumonia, septic shock, meningitis
Staphylococcus aureus		Furunculosis, cellulitis, abscess, septic shock, endocarditis
Coagulase-negative staphylococci		Infection of prosthetic devices, bacteraemia
Escherichia coli		Urinary tract infections, septic shock, haemorrhagic colitis
Klebsiella spp.		Urinary tract infections, septic shock, pneumonia
Enterobacter/ citrobacter		Urinary tract infections, pneumonia, septic shock
Pseudomonas aeruginosa		Urinary tract infections, pneumonia, septic shock
Neisseria meningitidis		Septic shock, meningitis
Haemophilus influenzae		Respiratory tract infections
Anaerobes		
Clostridium spp.		Tetanus, botulism, infections of soft tissue, abdominal sepsis, abscess
Peptococcus/ Peptostreptococcus spp.		Infections of soft tissue, abdominal sepsis, abscess
Bacteroides/Porphyromonas/Prevotella spp.		Infections of soft tissue, abdominal sepsis, abscess



7 The structure of the cell wall of gram-positive and gram-negative bacteria.

Table 2.3 Bacteria that are common causes of infections

Infections	Gram-negative pathogens	Gram-positive pathogens
Burns	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Skin infections		*S. aureus*
Throat		*Streptococcus pyogenes*
Otitis media	*Haemophilus influenzae*	*Streptococcus pneumoniae*
Pneumonia	*H. influenzae*	*S. pneumoniae*
Endocarditis		*S. aureus, Enterococcus faecalis*
Septicemia	*Escherichia coli*	*S. aureus, S. pyogenes*
Gastrointestinal tract	*Salmonella enterica* serovar Typhimurium *Helicobacter pylori, E. coli, Shigella dysenteriae*	
Urinary tract	*E. coli*	*Enterococcus* sp.

Adapted from Table 1.1 of Scholar and Pratt (2000), with permission.

Definitions:

Pneumonia: Inflammation of the lung, usually caused by bacteria or viruses.
Otis media: Inflammation of the middle ear
Endocarditis: Inflammation of the innermost tunic of the heart
Septicemia: Systemic disease caused by the spread of microorganisms and their toxins via the circulating blood (also called "blood poisoning")
Pathogen: a microorganism that causes disease.
Virulence: The disease-evoking severity of a pathogen

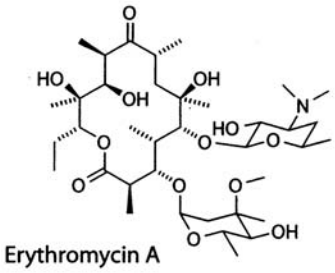
C. Classes of antibiotics

1. β-Lactam antibiotics
   examples: penicillins (e.g. amoxicillin), cephalosporins, carbapenems, monobactams, etc.
2. Tetracyclines
   example: tetracycline
3. Macrolide antibiotics
   example: erythromycin
4. Aminoglycosides
   examples: Gentamicin, Tobramycin, Amikacin
5. Quinolones
   example: Ciprofloxacin (a fluoroquinolone)
6. Cyclic peptides
   examples: Vancomycin, Streptogramins, Polymyxins
7. Lincosamides
   example: clindamycin
8. Oxazolidinoes
   example: Linezolid (Zyvox)
9. Sulfa antibiotics
   example: sulfisoxazole

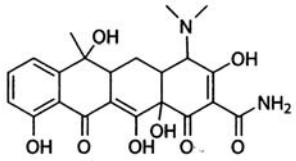
Antibacterial Drugs

Natural Products

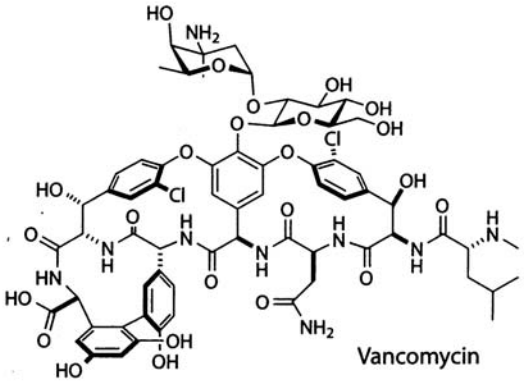
Amoxicillin



Erythromycin A

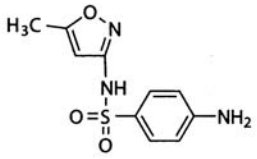


Tetracycline

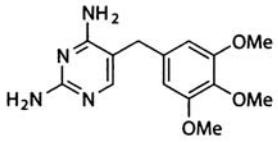


Vancomycin

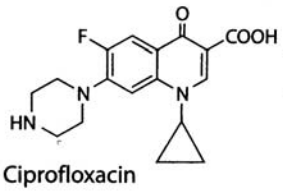
Synthetic Molecules



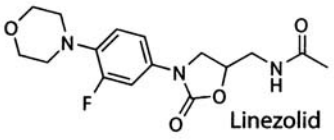
Sulfamethoxazole



Trimethoprim



Ciprofloxacin



Linezolid

Structures of naturally and synthetically derived antibacterials.

D. Which antibiotics are most commonly used?

Table 2.2 Antibiotic market in 1995

Class	Worldwide sales ($ millions)	Representative drugs	Infections that have developed resistance
Cephalosporins	8,446	Cefaclor, cefuroxime	Bronchitis, pneumonia, meningitis
Penicillins	4,413	Amoxicillin, ampicillin	Pneumonia, septicemia, bronchitis
Fluoroquinolones	3,309	Ciprofloxacin, ofloxacin	Toxic shock syndrome, meningitis
Macrolides	2,927	Clarithromycin, erythromycin	Toxic shock syndrome, meningitis
Tetracyclines	744	Minocycline	Urinary tract infections, pelvic inflammatory disease
Aminoglycosides	729	Gentamicin	Intestinal infections, septicemia
Glycopeptides	462	Vancomycin	Intestinal infections
All other systemic antibiotics	1,873	Imipenem, rifampin	Bronchitis, tuberculosis

Table 1.2 Summary of sales of major chemotherapeutic antibacterial agents

Class	Sales (billion of dollars)	Trend
Cephalosporins	6.0	up
Penicillins	2.5	little change
Quinolones	1.7	strongly up
Macrolides	1.5	slightly up
Tetracyclines	0.5	down
Aminoglycosides	0.5	down
Others	2.0	little change

Table 2.1 Antibiotic sales in 1997

Drug	$ millions
Cephalosporins	
Rocephin (Roche)	933
Ceftin (GlaxoWellcome)	640
Ceclor (Lilly)	542
Fortaz (GlaxoWellcome)	449
Claforan (Hofmann LaRoche)	335
Macrolides	
Biaxin (Abbott)	1,150
Zithromax (Pfizer)	619
β-Lactamase inhibitors	
Augmentin (GlaxoSmithKline)	1,354
Primaxin (Merck)	555
Unasyn (Pfizer)	619
Penicillins	
Amoxil (GlaxoSmithKline)	406
Quinolones	
Ciprofloxacin (Bayer)	1,290

How do antibiotics work?

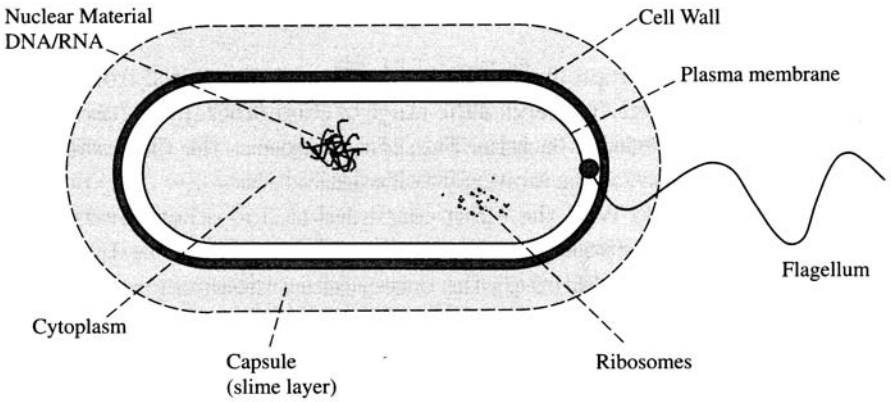


Fig. 14.4 The bacterial cell.

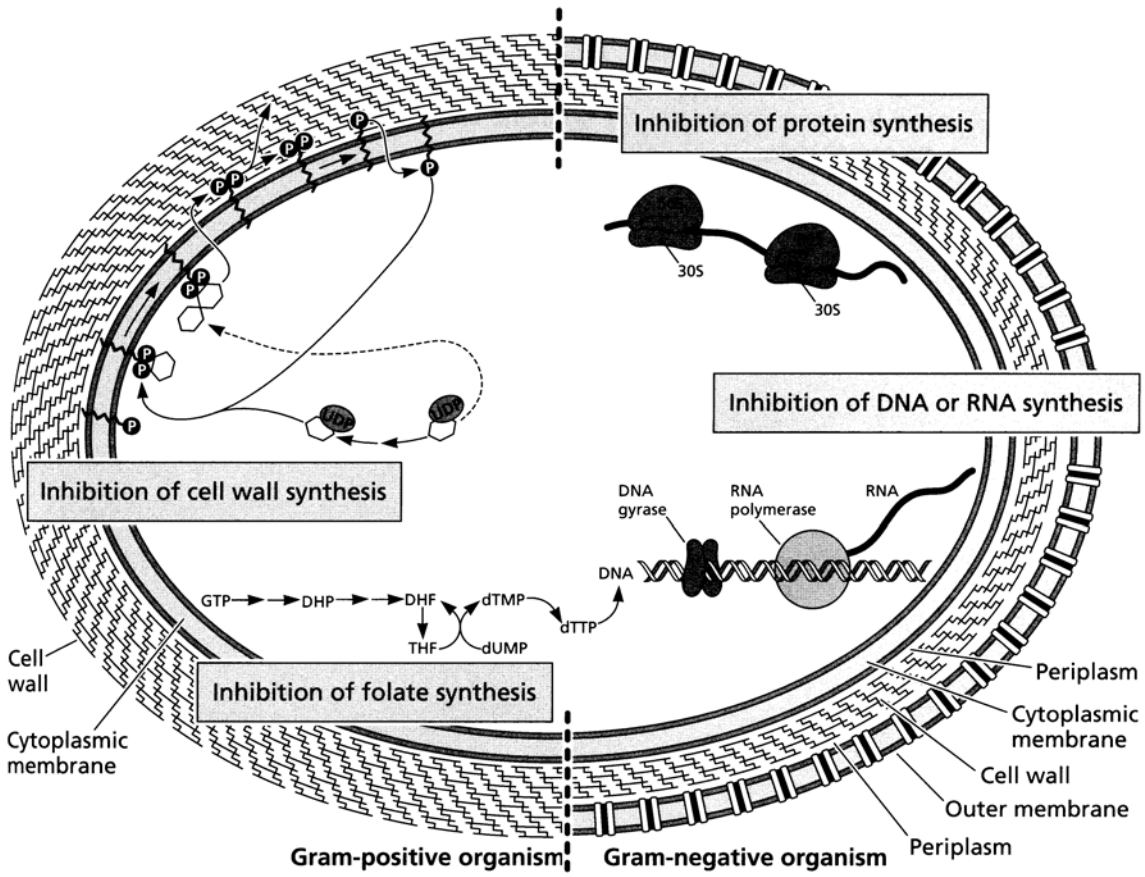


Figure 2.2 Major targets for antibacterial action. (Adapted from a poster on Mechanisms of Antibiotic Action and Resistance, C. Walsh, J. Trauger, P. Courvalin, and J. Davies [2001], *Trends in Microbiology, The Lancet Infectious Disease, Current Opinion in Microbiology, Trends in Molecular Medicine.*)

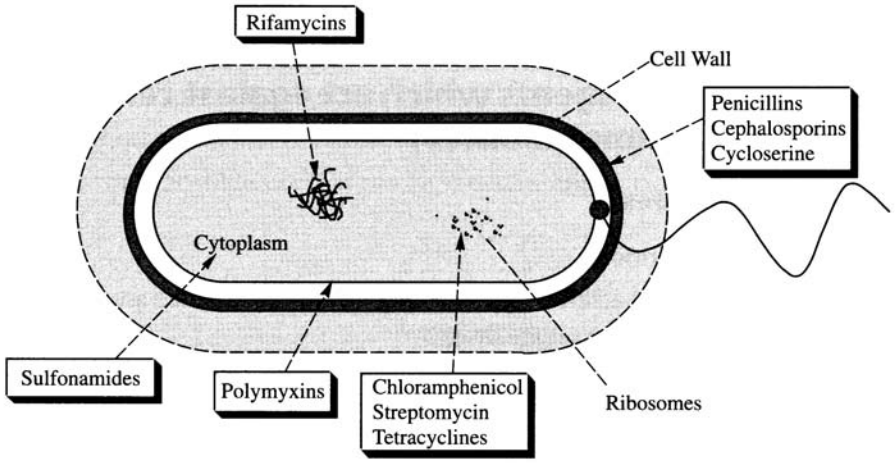


Fig. 14.5 Sites of antibacterial action.

Table 2.5 Major antibiotics: structural classes, targets, and resistance mechanisms

Antibiotic	Target	Resistance mechanism
Cell wall		
β-Lactams	Transpeptidases/ transglycosylases (PBPs[1])	β-Lactamases, PBP mutants
Vancomycin Teicoplanin	D-Ala-D-Ala termini of peptidoglycan and of lipid II	Reprogramming of D-Ala-D-Ala to D-Ala-D-Lac or D-Ala-D-Ser
Protein synthesis		
Erythromycins	Peptidyltransferase/ribosome	rRNA methylation/efflux
Tetracyclines	Peptidyltransferase	Drug efflux
Aminoglycosides	Peptidyltransferase	Drug modification
Oxazolidinones	Peptidyltransferase	Unknown
DNA replication/repair		
Fluoroquinolones	DNA gyrase	Gyrase mutations

[1]PBP, penicillin-binding protein.

Why is there a need for new antibiotics?

Table 1.1 Evolution of resistance to antibiotics

Antibiotic°	Year deployed	Resistance observed
Sulfonamides	1930s	1940s
Penicillin	1943	1946
Streptomycin	1943	1959
Chloramphenicol	1947	1959
Tetracycline	1948	1953
Erythromycin	1952	1988
Vancomycin	1956	1988
Methicillin	1960	1961
Ampicillin	1961	1973
Cephalosporins	1960s	late 1960s

From Palumbi (2001), with permission.

Table 1.1 Bacteria that have gained resistance to some drug therapy

Bacteria	Disease/disorder	Date (approx.)
Penicillin resistant		
Pneumococci	Pneumonia, meningitis	mid 1970s–present
Legionella	Legionnaire's disease (pneumonia)	mid 1970s–present
Borrelia burgdorferi	Lyme disease	1980s–present
Salmonella	Gastrointestinal disorders	1980s–present
Staphylococci	Toxic Shock Syndrome	1980s
E. coli O157:H7	Gastrointestinal disorders	mid 1980s–present
Multi-drug resistant		
M. tuberculosis	Tuberculosis	late 1980s–present
Vancomycin resistant		
Enterococci	Wound, blood and enteric infections	late 1980s–present
V. cholerae	Cholera	present
Multi-drug resistant 'super bugs'		?????

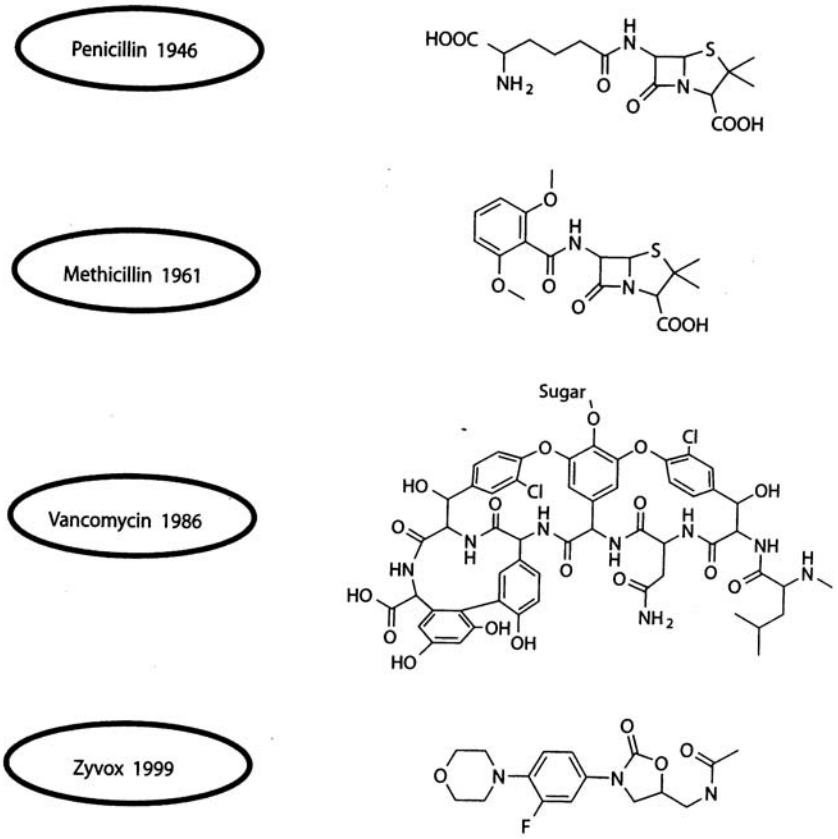


Figure 1.2 Progression of antibiotics required for efficacy in staphylococcal infections. (Adapted from Palumbi [2001], with permission.)

Why does resistance develop?

The large numbers of bacterial cells, combined with the short generation times facilitate the development of mutants. In a typical bacterial population of 10^{11} bacterial cells (e.g. in an infected patient) there can easily be 1000 mutants. If a mutant confers a selective advantage upon the bacterium (e.g. the ability to survive in the presence of an antibiotic) then that resistant bacterium will be selected

and continue to grow while its neighbors perish. This can happen in a matter of days in patients being treated with antibiotics.

B. Origins of antibiotics

1. Most classes of antibiotics, including the b-lactam antibiotics, tetracyclines, aminoglycosides, and macrolides. originally derived from natural sources, and were then further chemically modified to confer better properties on the drug.

2. However, some important classes of antibiotics (including the sulfa antibiotics, the quinolones, and the oxazolidinones) are man-made, originating totally from synthetic chemical operations.

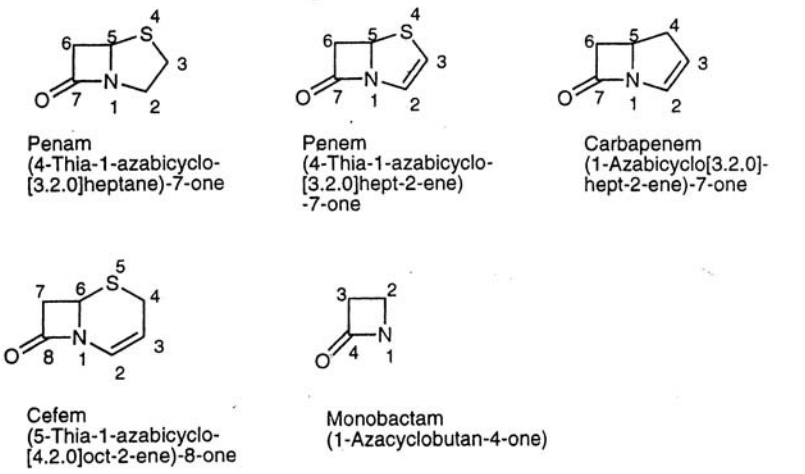
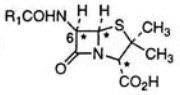


Fig. 34.9. Ring and numbering systems of clinically available β-lactam antibiotic types.

Table 34.3. Commercially Significant Penicillins and Related Molecules



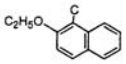
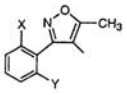
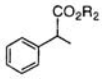
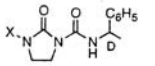
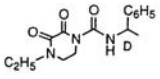
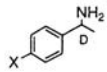
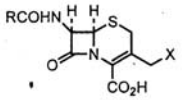
Generic Name	Trade Name	R_1	X	Y
Fermentation-Derived Penicillins				
6-Aminopenicillanic acid		H	—	—
Benzylpenicillin (Penicillin G)	Generic	C_6H_5-CH_2-	—	—
Phenoxymethylpenicillin (Penicillin V)	Generic	C_6H_5-OCH_2-	—	—
Semi-Synthetic Penicillinase-Resistant Parenteral Penicillins				
Methicillin			—	—
Nafcillin	Nallpen, Unipen		—	—
Semi-Synthetic Penicillinase-Resistant Oral Penicillins				
Oxacillin	Bactocill		H	H
Cloxacillin	Cloxapen		H	Cl
Dicloxacillin	Dycil, Pathocil		Cl	Cl
Semi-Synthetic Penicillinase-Sensitive, Broad Spectrum, Parenteral Penicillins				
Carbenizillin (R_2 = H)			—	—
Carbenicillin phenyl (R_2 = C_6H_5)				
Carbenicillin indanyl (R_2 = )	Geocillin			
Ticarcillin	Ticar		—	—
Azlocillin	Mezlin		H	—
Mezolcillin			CH_3SO_2-	—
Piperacillin	Pipracil		—	—
Semi-Synthetic Penicillinase-Sensitive, Broad-Spectrum, Oral Penicillins				
Ampicillin	Principen, Omnipen		H	—
Amoxicillin	Amoxil, Trimox, Wymox		HO	—

Table 34.8. First-generation Cephalosporins



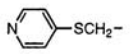
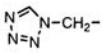
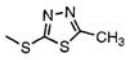
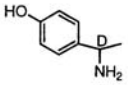
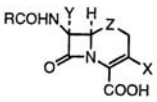
Generic Names	Trade Names	R	X	Salt
Parenteral Agents:				
Cephapirin	Cefadyl		OAc	Na
Cefazolin	Ancef, Kefzol, Zolicef			Na
Oral Agents:				
Cephalexin	Keflex, Biocef Keftab		H	HCl
Cefadroxil	Duricef		H	—
Oral and Parenteral Agents:				
Cephradine	Velosef		H	—

Table 34.9. Second-generation Cephalosporins



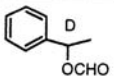
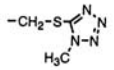
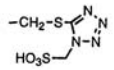
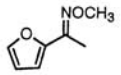
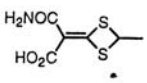
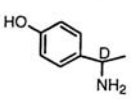
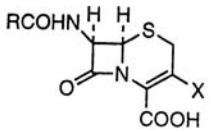
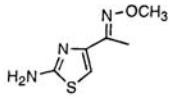
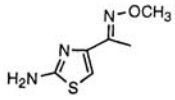
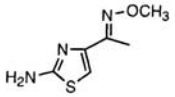
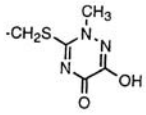
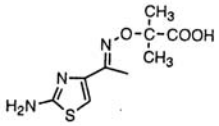
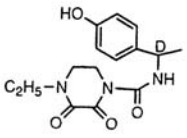
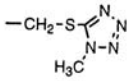
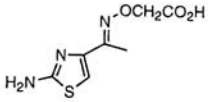
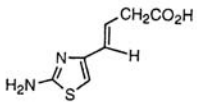
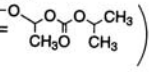
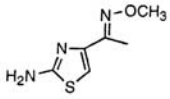
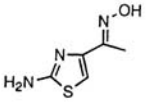
Generic Name	Trade Name	R	X	Y	Z	Salt
Parenteral Agents						
Cefamandole nafate	Mandol			H	S	—
Cefonicid	Monocid			H	S	diNa
Cefuroxime	Ceftin Kefurox Zinacef		$-CH_2OCONH_2$	H	S	Na
Cefoxitin	Mefoxin		$-CH_2OCONH_2$	OCH_3	S	Na
Cefotetan	Cefotan			OCH_3	S	diNa
Oral Agents						
Cefaclor	Ceclor		Cl	H	S	—
Loracarbacef	Lorabid		Cl	H	CH_2	—
Cefprozil	Cefzil			H	S	—

Table 34.10. Third-generation Cephalosporins



Generic Name	Trade Name	R	X	Salt
Parenteral Agents				
Cefotaxime	Claforan		CH_2OAc	Na
Ceftizoxime	Cefizox		H	Na
Ceftriaxone	Rocephin			diNa
Ceftazidime	Fortaz Ceptax Tazidime Tazicef			H or Na
Cefoperazone	Cefobid			Na
Oral Agents				
Cefixime	Suprax		$-HC=CH_2$	—
Ceftibuten	Cedax		H	—
Cefpodoxime proxetil (2-carboxyester= )	Vantin		$-CH_2OCH_3$	—
Cefdinir	Omnicef		$-HC=CH_2$	—

I. Penicillins
   A. History
      1. 1928: Alexander Fleming noticed killing effect of mold accidentally blown onto his agar plate. After attempt at isolation of compound responsible, judged to be too unstable for use as antibiotic
      2. 1938, Problem of isolating penicillin solved by Florey and Chain using a process called "freeze drying" now called lyophilization.
      3. 1941, first clinical trial of penicillin were successful
      4. 1944, used against casualties in D-day landing
      5. 1945, structure of penicillin finally solved
   B. Show structure of Benzylpenicillin (Penicillin G)
      1. Structure was solved by x-ray crystallography by Dorothy Hodgkins
      2. Previous to this, such a structure was proposed but was said to be "impossibly strained"
   C. Key features of structure
      1. β-lactam ring
         a. "Lactam" is a word for any cyclic amide (the word "lactone" is used for a cyclic ester)
         b. a β-lactam means that the nitrogen is joined to the carbon which is beta to the carbonyl
         c. this creates strain in the ring, since it is a four membered ring
         d. b-lactam becomes good acylating agent for active site serine of penicillin binding protein (see later)
      2. Carboxylate
         a. Negatively charged at neutral pH
         b. Anchors drug in active site pocket (positively charged)
      3. Acylamido side chain
         a. Necessary for biological potency
         b. Proper stereochemistry of attachment to ring essential for activity
         c. Variation at side chain can dramatically affect biological activity against various strains of bacteria
   D. Common Early Penicillins
      1. Penicillin G had to be administered parenterally, since it is not acid stable
      2. Penicillin V has more acid stability, and can be administered orally

How Does Penicillin Work?

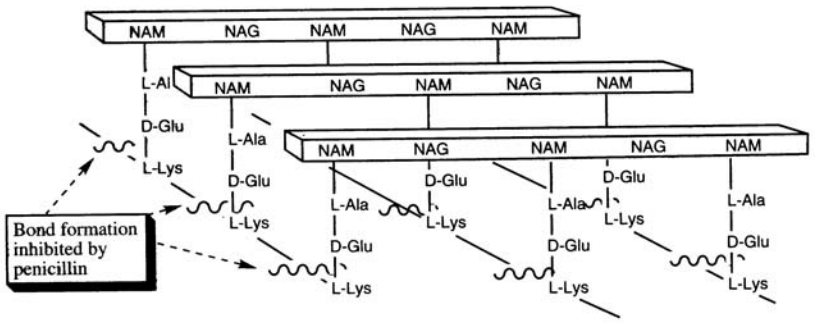


Fig. 14.60 Peptidoglycan structure.

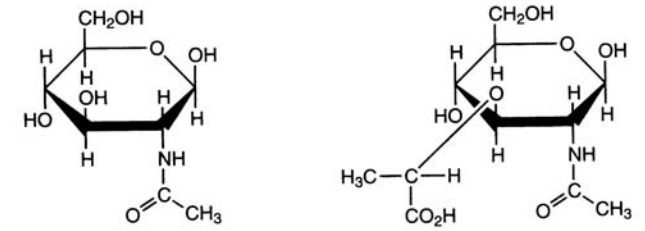


Fig. 14.61 Sugars contained in cell wall structure of bacteria.

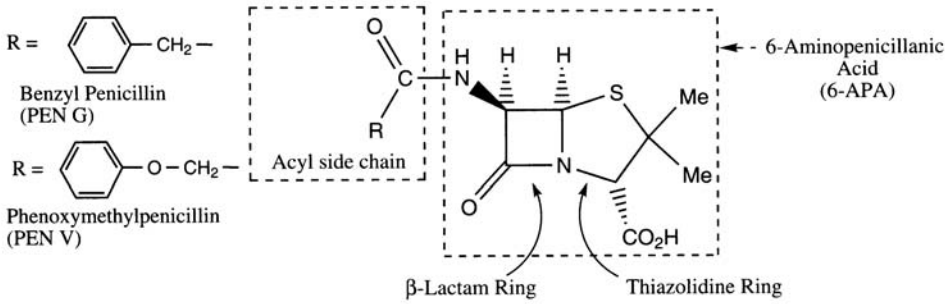


Fig. 14.18 The structure of penicillin.

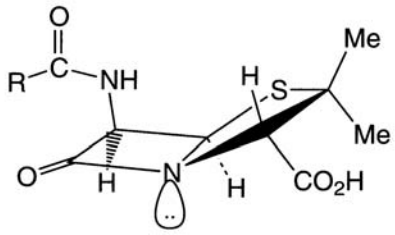


Fig. 14.20 Shape of penicillin.

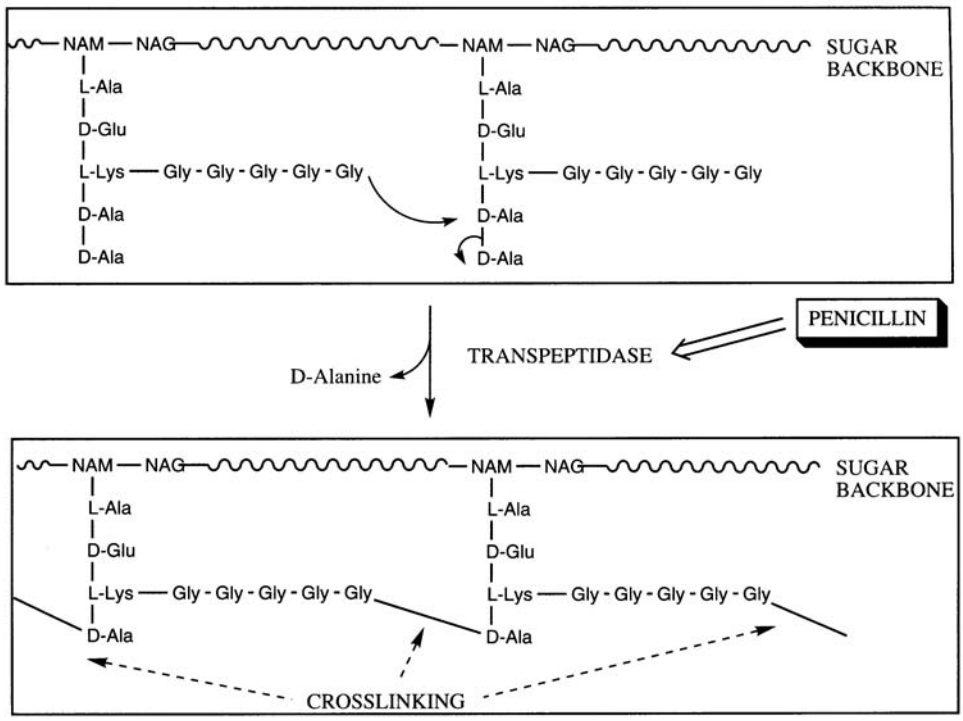


Fig. 14.62 Cross-linking of bacteria cell walls inhibited by penicillin.

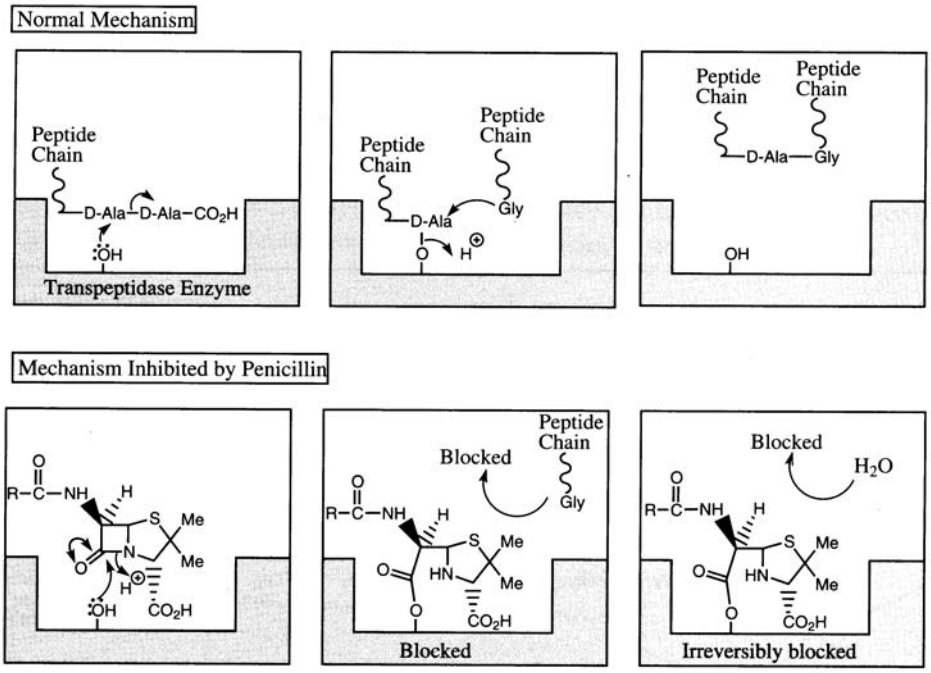


Fig. 14.63 Cross-linking mechanism by transpeptidase enzyme.

II. Penicillin Resistance due to β-Lactamase
   A. What is β-lactamase?
   B. Why is it a problem?

III. Penicillins: Part II

A. Methicillin: A drug designed to be resistant to b-lactamase (previously called penicillinase).

1. Structure of methicillin
2. Notice "steric shield" on side chain to protect b-lactam from hydrolysis
3. Biological activity & pharmacokinetics of methicillin
   a. Has to be administer parenterally, since it has no electron withdrawing group on the side chain
   b. Inactive against gram negative bacteria

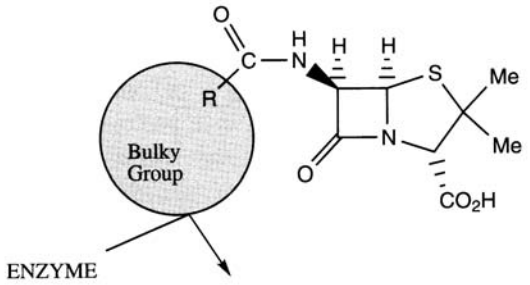


Fig. 14.31 Blocking penicillin from reaching the penicillinase active site.

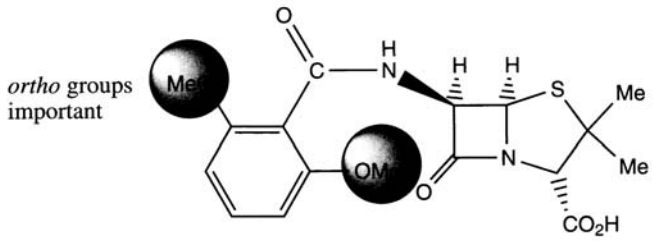


Fig. 14.32 Methicillin.

B. Oxacillin

1. Still resistant to b-lactamases
2. More acid stability than methicillin

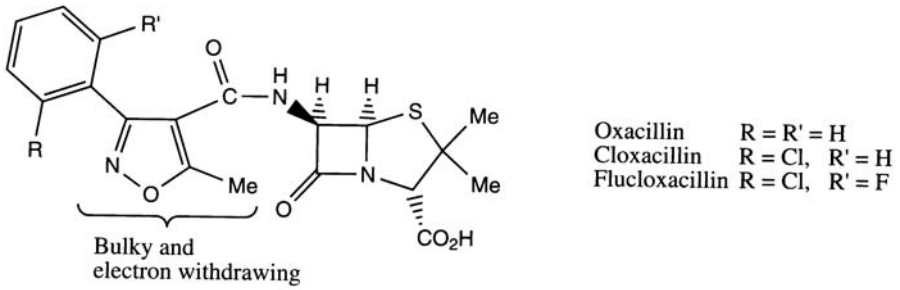


Fig. 14.33 Incorporation of a five-membered heterocycle.

C. Better Gram-negative activity: Ampicillin and Amoxicillin
   1. Attaching a hydrophilic group to the side chain seemed to give the drug better Gram negative activity
   2. This was achieved by employing an amino substituent directly adjacent to carbonyl of side chain
   3. Still inactive agains Pseudomonas aeruginosa, a particularly challenging pathogen
   4. Sometimes administered as prodrugs (esters) due to poor absorption through the gut (show pivampicillin structure)

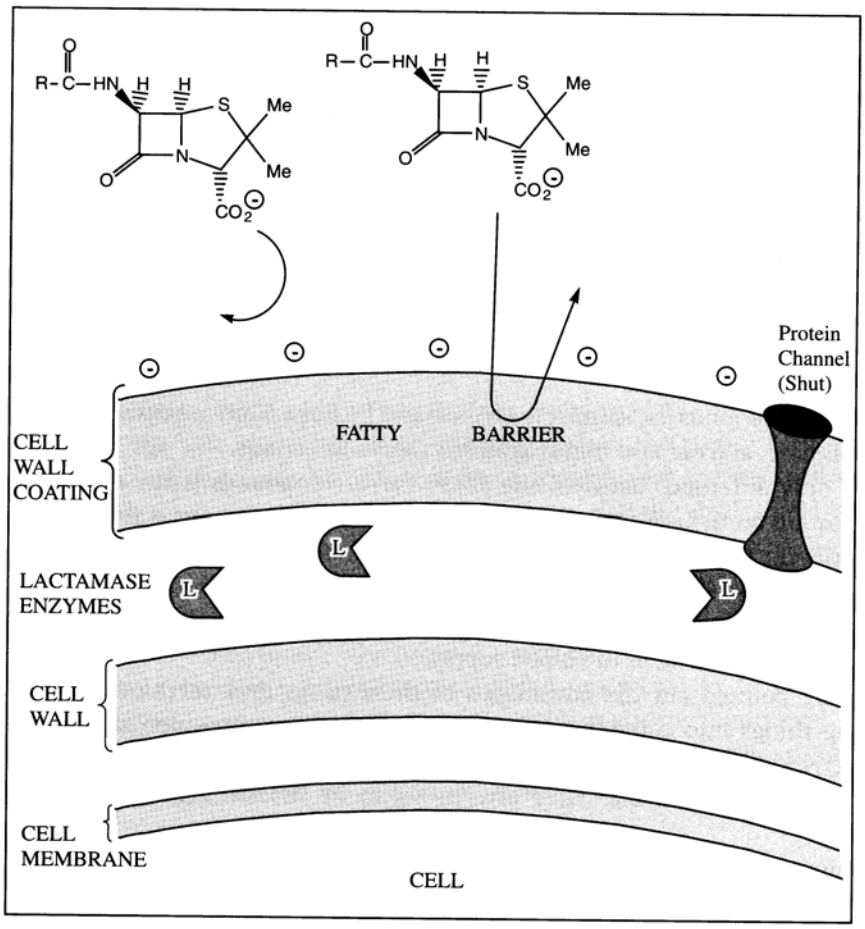
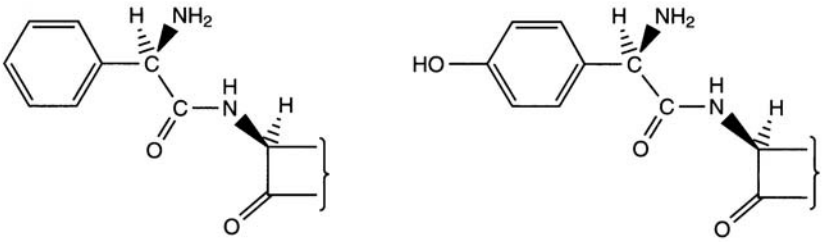


Fig. 14.34 Permeability barrier of a Gram-negative bacterial cell.



AMPICILLIN (Penbritin) AMOXYCILLIN (Amoxil)

Fig. 14.36 Class I broad spectrum antibiotics.

D. Best activity against Gram-negative organisms, including Pseudomonas aeuruginosa: carbenicillin

II. Cephalosporins:

A. History

1. First isolated from fungus found in sewer line on island of Sardina in 1948
2. Structure wasn't elucidated until 1961

B. Prototypical Early cephalosporin: Cephalothin

1. Less antibiotic activity than Penicillin G against Gram positive bacteria
2. More activity than Pen G against Gram negative bacteria
3. Can be used on patients who are allergic to penicillin
4. Side chain acetoxy group is hot point for metabolic inactivation

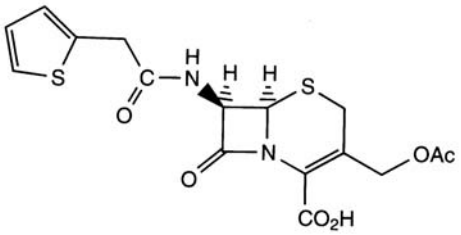


Fig. 14.46 Cephalothin.

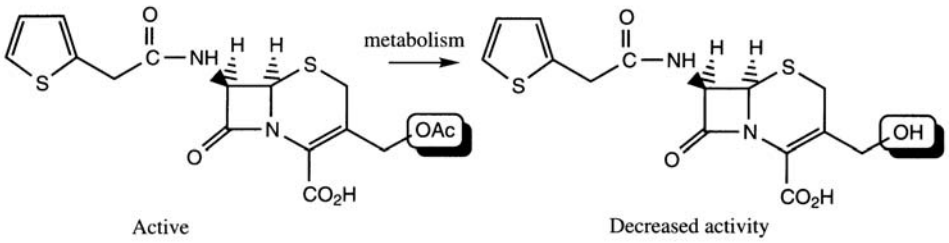


Fig. 14.47 Metabolic hydrolysis of cephalothin.

C. Cephaloridine

1. Better leaving group in form of positively charged pyridinium group will "activate" system
2. Avoids metabolic inactivation

3. Note that compound is "zwitterion"

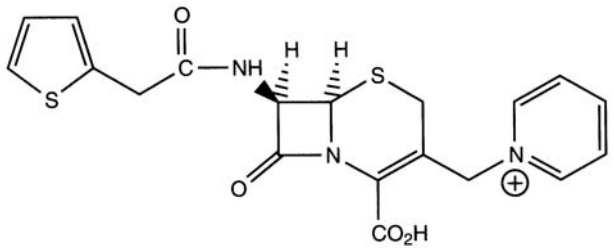


Fig. 14.48 Cephaloridine.

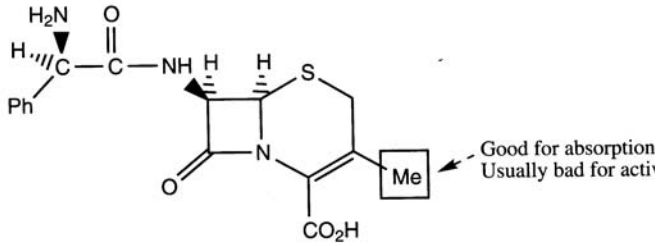


Fig. 14.49 Cephalexin.

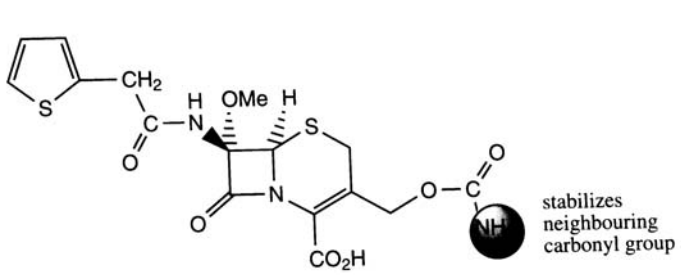


Fig. 14.52 Cefoxitin.

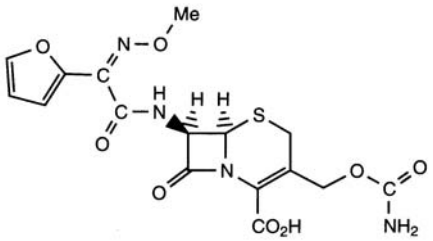


Fig. 14.53 Cefuroxime.

D. Ceftazidime
   1. Combines activation of ceftazidime with steric shielding of b-lactam to protect it from hydrolysis by b-lactamase
   2. Note additional hydrophilic groups on side chain further improve activity against gram negative strains.

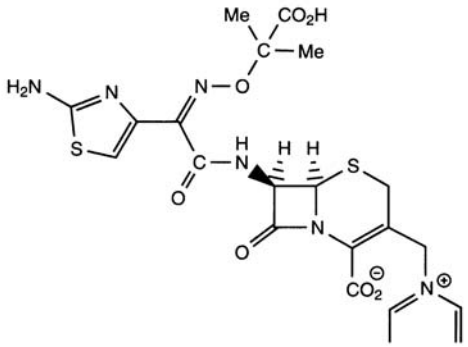


Fig. 14.54 Ceftazidime.