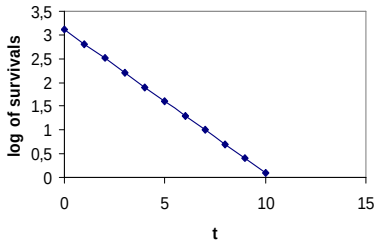
Sterilization	kill / remove all microorganisms
Disinfection	kill many but not all microorganisms
Antiseptics	disinfectants applicable on skin or mucous membranes

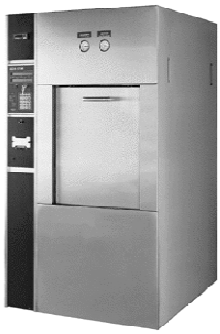
Antimicrobial kinetics



$S = S_0 \times e^{-kt}$
-k: death rate
S_0: germ number at start
S: germ number at time t

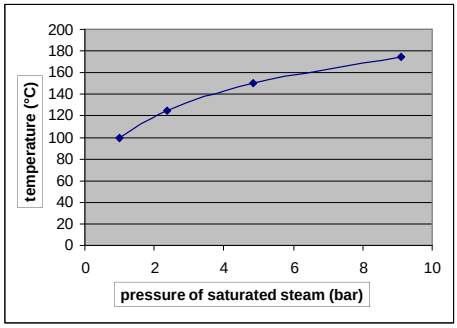
Heat sterilization

Autoclave – moist heat



Hot-air oven – dry heat

Physical principal of autoclaving



Antimicrobial heat treatment methods

(temperature & time)

Heat sterilization:

Moist heat - autoclave:
saturated steam at 1 bar overpressure (121°C) & 30 min

Dry heat - hot-air oven:
160 °C or more
time≥30 min

burning, heating to re

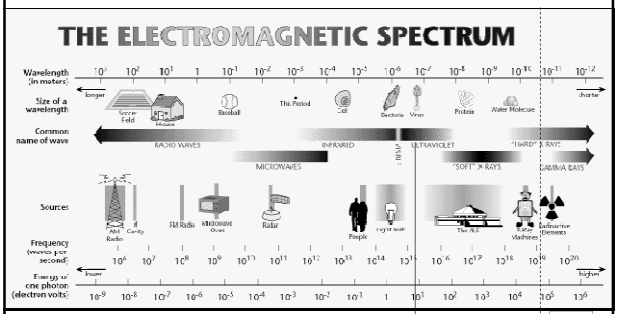
Reverse relationship with temperature
with circulation: e.g. 160 °C 45 min, 180 °C 30 min
without circulation: e.g. 160 °C 2 hours, 180 °C 1,5 hours

Non-sterilizing treatment methods:

Pastuerizing milk, food
62-95°C & 30-1 minutes

Fractionated sterilization:
80°C & 30 min on 3 consecutive days
e.g. hepatitis B virus can survive

Using electromagnetic irradiation



At 254 nm wavelength it damages DNA but it can exert its effect only on surfaces & in air

not for sterilization, rather for sanitation

reliable sterilization

Antimicrobial effect of ionizing radiation

Radiolysis of water

$H_2O \Rightarrow H_2O^+ + e^-$

$H_2O^+ + H_2O \Rightarrow H_3O^+ + \cdot OH$

$e^- + H_2O \Rightarrow OH^- + H\cdot$

Application:
disposable plasticware, needles

free radicals reacting with macro-molecules

Filtration

For air and heat sensitive fluids
but not for those of live origin (due to viruses)

Fluids
Membrane filters: nitrocellulose etc.
Standard pore size (0,22 μm, 0,45 μm)

Air
HEPA filter (High efficiency particulate air)
For sterile compartments (laminar air flow cabinets)

Determinants of disinfection

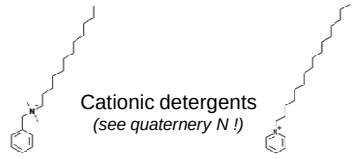
- Chemical composition & concentration
- Time of exposure
- Temperature
- pH
- Entity of microorganism
  (bacterium, spore, fungus, virus, parasite)
- Contaminants (blood, feces, mucilage, etc.)

•***Basis of classification is chemical structure !***

1. Detergents (tensids)

Amphoter molecules – damage to cell membrane

- Antimicrobial spectrum:
  bacteria, fungi, (some viruses)



Cationic detergents
(see quaternery N !)

Benzalkonium chloride

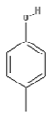
Cetylpyridinium chloride

•*Anionic detergents (soaps) have little if any antimicrobial effect*

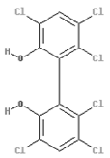
2. Phenols

Damage to cell membranes, denaturing proteins (phenol), cresol, hexachlorophen, chlorohexidine

Antimicrobial spectrum:
bacteria, (some fungi, viruses)



Para-cresol



Hexachlorophene

3. Alcohols
Damage to cell membranes, denaturing proteins
Ethanol, propanol, iso-propanol
Antimicrobial spectrum: bacteria, (some fungi, viruses)

4. Chlorines (oxydizing agent)
Oxydizing free –SH groups of enzymes,structure proteins
Sodium-hypochlorite (NaOCl), $Ca(OCl)_2$
Antimicrobial spectrum: bacteria & spores, viruses, (some parasites)

5. Iodines (oxydizing agent)
Damage to cell membranes, denaturing proteins
Antimicrobial spectrum: bacteria, fungi, (some viruses)

6. Heavy metals: mercury, arsene, silver compounds

Alkylating agents: broadest antimicrobial spectrum

7. Aldehydes
Cross-linking proteins, nucleic acids
Formaldehyde, glutaraldehyde

8. Gas sterilization in chamber
Ethylenoxide, beta-propio-lakton

ethylenoxide

beta-propio-lakton

	antimicrobial drug	**disinfectant, antiseptic**
	exploits differences between the function and structure of the host and the microbe	*similar effect upon live cells with wide range of origin*
	SELECTIVE TOXICITY	**NOT SELECTIVE**
	utility	
systemic application	yes	**NEVER**
topical application	yes	limited
environmental sanitation	no sense *emerging resistance!*	yes

Drugs against infections

Requirements:

•sufficient therapeutic concentration and appropriate dosage and timing
(see Pharmacology)

•efficacy against the etiological (causative) agent
(see Medical Microbiology)

•RANGE OF ANTIBACTERIAL ACTIVITY
broad spectrum – narrow spectrum

Major groups of pathogenic bacteria used for characterization of antibacterial spectrum

non-fermentative Gram– rods	**fermentative Gram–b.**	**Gram+ bacteria**	**obligate intracellular & cell wall defective**	**Mycobacteria**
e.g. **Pseudomonas**	***e.g.*** **E. coli Salmonella**	***e.g.*** **Staphylo-coccus**	***e.g.*** **Chlamydia Mycoplasma**	***e.g.*** **causative agents of tuberculosis**

•ANTIBACTERIAL EFFECT
bactericidal – bacteriostatic

Cell wall inhibitors	beta-lactams, vancomycin, cycloserine, bacitracin
Protein synthesis inhibitors	erythromycins, clindamycin, aminoglycosides, tetracyclins, chloramphenicol, linezolid, streptogramins
Nucleic acid synthesis inhibitors	quinolones, sulfonamides, trimethoprim, rifampin
Alteration of cell membrane function	polymyxins
Miscellaneous	metronidazole, isoniazid, ethambutol

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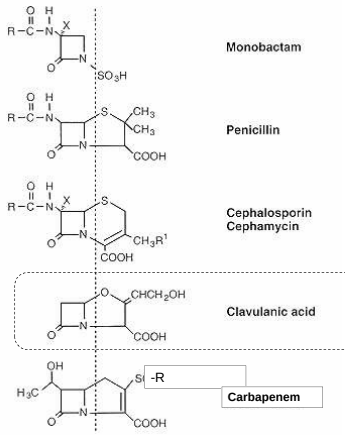
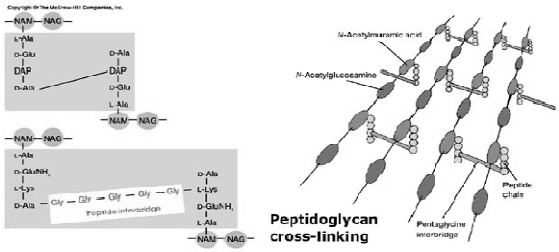


Beta-lactams

Structural analogues of peptide side chain in peptidoglycan

not antibacterial ! see later

Peptidoglycan structure



Beta-lactams mechanism of action

- structural analogue of D-Ala-D-Ala in the peptide side chain
- interferes with **proteins** involved in the peptidoglycan pathway
- target: **Penicillin binding proteins (PBP)**
- inhibiton of cell wall synthesis – blocks bacterial replication
- osmotic lysis of bacterial cells with altered cell wall structure

- induction of bacterial autolysins (peptidoglycan hydrolases)

Bactericidal antibiotics

ANTIBACTERIAL SPECTRUM

Pseudomonas	fermentative Gram– b.	Gram+ bacteria	obligate intracellular & cell wall defective	Mycobacteria
Penicillins *sensitive to bacterial beta-lactamase:*				
		penicillin-G	NONE	
	aminopenicillins (amoxicillin)			
ureidopenicillins (piperacillin)				
Penicillins *resistant to bacterial beta-lactamase*				
		methicillin nafcillin	NONE	
	amoxicillin+ clavulanic acid			
piperacillin+ tazobactam				

ANTIBACTERIAL SPECTRUM

Pseudomonas	fermentative Gram– b.	Gram+ bacteria	obligate intracellular & cell wall defective	Mycobacteria
Cephalosporins *sensitive to the beta-lactamases of certain Gram-negatives*				
		1st generation	NONE	
	2nd generation			
Cephalosporins *resistant to most types of Gram-negative beta-lactamases (not ESBL)*				
parenteral 3rd gen	3rd generation		NONE	
4th generation				
Carbapenems *are resistant to the beta-lactamase of most bacteria*				
imipenem, meropenem			NONE	

BACTERIAL RESISTANCE AGAINST BETA-LACTAMS

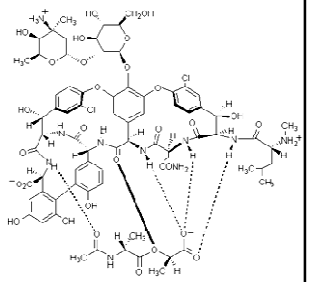
1. **Beta-lactamase** production: inactivates penicillins, cephalosporins or both
2. **Altered penicillin-binding proteins** -target is changed
3. **Poor permeability**- *through the Gram-neg. outer membrane*
4. **Tolerance** - m*utant autolysins are not activated*

Example for resistance to beta lactams:Staphylococcus aureus (Gram +)

1.beta-lactamase *penicillinase only*	2. altered PBP e.g. **MRSA !**	4. tolerance
RESISTANT TO		
e.g. pen-G, amox, pip	**all beta-lactams !**	
SENSITIVE TO		
beta-lactamase resistant penicillins, 1st gen. cephalosporins, carbapenems	vancomycin	same as non-tolerant but the bacteria are not killed

Glycopeptides

- Target: pentapeptide in transpeptidation
- Mode of action: inhibits cell wall synthesis
- Spectrum of effect: narrow, Gram positives
- Bactericidal drugs
- **Vancomycin** and teicoplanin

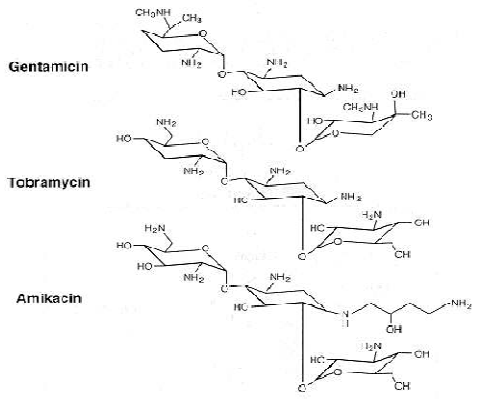


Cell wall inhibitors	beta-lactams, vancomycin, cycloserine, bacitracin
Protein synthesis inhibitors	erythromycins, clindamycin, aminoglycosides, tetracyclins, chloramphenicol, linezolid, streptogramins
Nucleic acid synthesis inhibitors	quinolones, sulfonamides, trimethoprim, rifampin
Alteration of cell membrane function	polymyxins
Miscellaneous	metronidazole, isoniazid, ethambutol

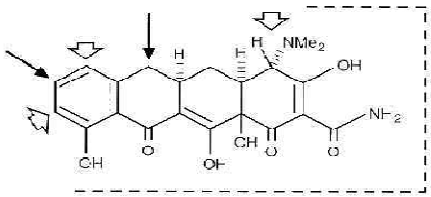
ANTIBACTERIAL SPECTRUM

Pseudomonas	**fermentative Gram– b.**	**Gram+ bacteria**	**obligate intracellular & cell wall defective**	**Mycobacteria**
aminoglycosides *amikacin > gentamicin = tobramycin*				streptomycin *ototoxic !*
	**not recommended b-static*		**tetracyclines**	
				erythromycins / macrolids *only certain Gram-negatives are sensitive * atypical Mycobact

Aminoglycosides



Tetracycline

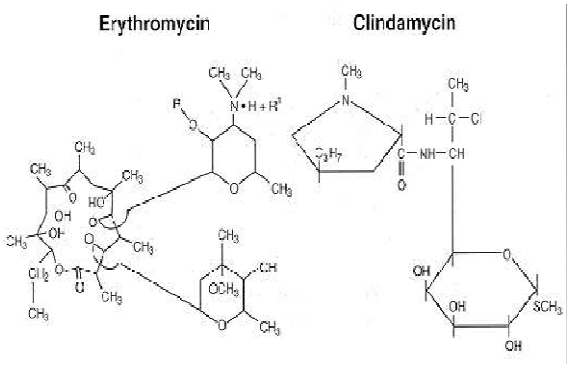


Critical Parts of the Molecule

Sites of major modification

Secondary modification sites

Area critical for act vity



ANTIBACTERIAL SPECTRUM

Pseudomonas	fermentative Gram– b.	Gram+ bacteria	obligate intracellular & cell wall defective	Mycobacteria
		Clindamycin +some Gr.-neg anaerobes		
	chloramphenicol *not recommended, toxic!*			

Streptogramins (quinupristin/dalfopristin) and linezolid are used presently against Gram positive cocci

Bactericidal: aminoglycosides, streptogramins

Bacteriostatic: tetracyclins, linezolid, clindamycin, erythromycin, chloramphenicol, *can be bactericidal to certain bacteria*

MECHANISMS OF BACTERIAL RESISTANCE

aminoglycosides	•biochemical conjugation •mutation of the target protein (on 30 S subunit) •poor permeability
tetracyclines	•reduced intracellular uptake •enhanced efflux
macrolids	•methylate 23 S RNA (on 50 S subunit)
chloramphenicol	•biochemical conjugation

Cell wall inhibitors	beta-lactams, vancomycin, cycloserine, bacitracin
Protein synthesis inhibitors	erythromycins, clindamycin, aminoglycosides, tetracyclins, chloramphenicol, linezolid, streptogramins
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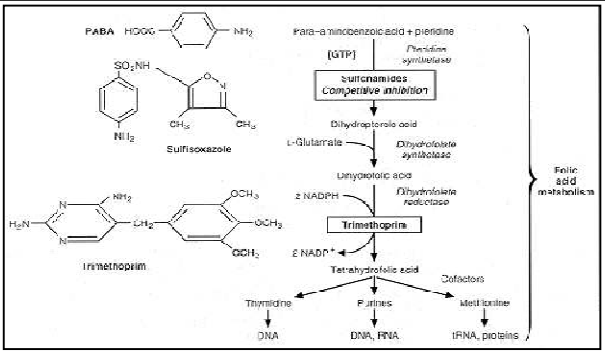

sulfonamide	**-**	*see above*	**- bacteriostatic**
		together with trimethoprim	**- bactericidal**
quinolones	**-**	**inhibit bacterial DNA-gyrase**	**– *bactericidal***
rifampin	**-**	**block bacterial mRNA synthesis-**	***bactericidal***

ANTIBACTERIAL SPECTRUM

Pseudomonas	fermentative Gram– b.	Gram+ bacteria	obligate intracellular & cell wall defective	Mycobacteria
	sulfonamide + trimethoprim			
	quinolones 1st generation			
	fluoroquinolones			
e.g. ciprofloxacin (3rd gen)	2nd – 4th gen.	3rd – 4th gen.	2nd – 4th gen.	ofloxacin (2nd g) ciprofloxacin (3rd)
	rifampin **in case of polyresistant b.*			**rifampin**

Principals of antibiotic chemotherapy

1. Uncomplicated bacterial infections **monotherapy** (select the best!)

there is time for antibiotic testing	no time is left for antibiotic testing
•use specific therapy based on the antibiogram	•use the drug that probably covers the most of the possible pathogens •*first choice* guidelines e.g. respiratory tract inf. - macrolids

2. Life-threatening bacterial infections
use **broad spectrum, bactericidal** antibiotics – single or in combination

3. Chronic bacterial infections & slowly replicating bacteria (e.g. tbc)
usually require combined antibiotic therapy
reason: during slow replication bacteria are less responsive even against an effective antibiotic - more risk of developing resistance to a single drug