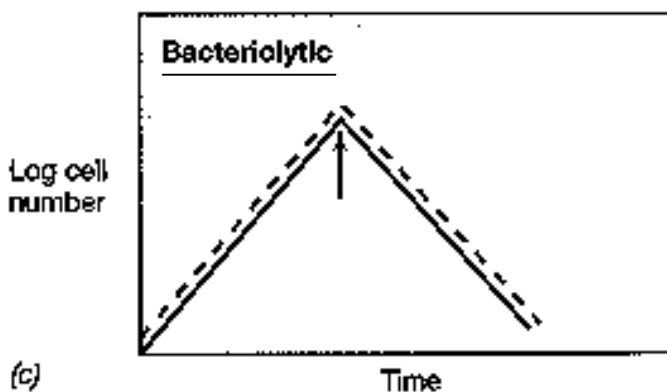
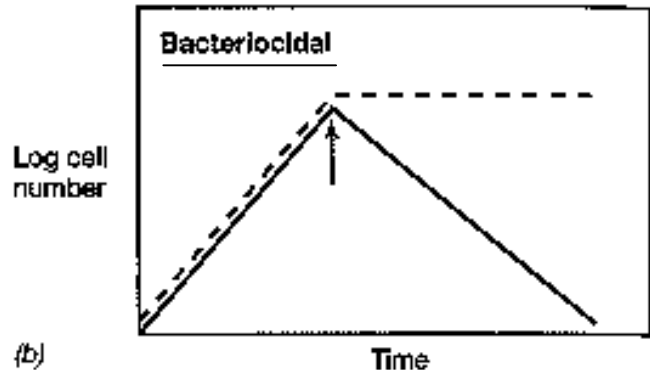
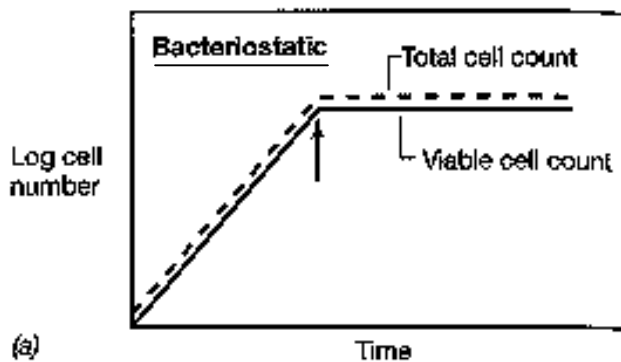


## MMG 301 Dr. Frank Dazzo

### Control of Microbial Growth: Chemical Antimicrobials

- Previously described physical factors (e.g., temperature) affecting microbial growth. Here focus on chemical antimicrobials affecting microbial growth.
- Two main categories of antimicrobial agents:
  - non-chemotherapeutic agents: (e.g., antiseptics, disinfectants); used to inhibit or kill microbes on living surfaces (topically) or inanimate objects, but are too toxic for internal use inside the human body.
  - chemotherapeutic agents: (growth factor analogs, antibiotics) can be used internally to control microbes that cause infectious diseases.
- Based on degree of selective toxicity to the human or domestic animal host.
- Some Important (suffix) Terminology:
  - “-static”: antimicrobials that inhibit microbial growth without killing them, e.g., bacteriostatic, fungistatic.
  - “-cidal”: antimicrobials that kill microbes, e.g., bacteriocidal, fungicidal.
  - “-lytic”: antimicrobials that kill microbes by lysing them, e.g., bacteriolytic.

Distinguish these 3 different categories by examining the effect of the antimicrobial agent on a growing bacterial culture:



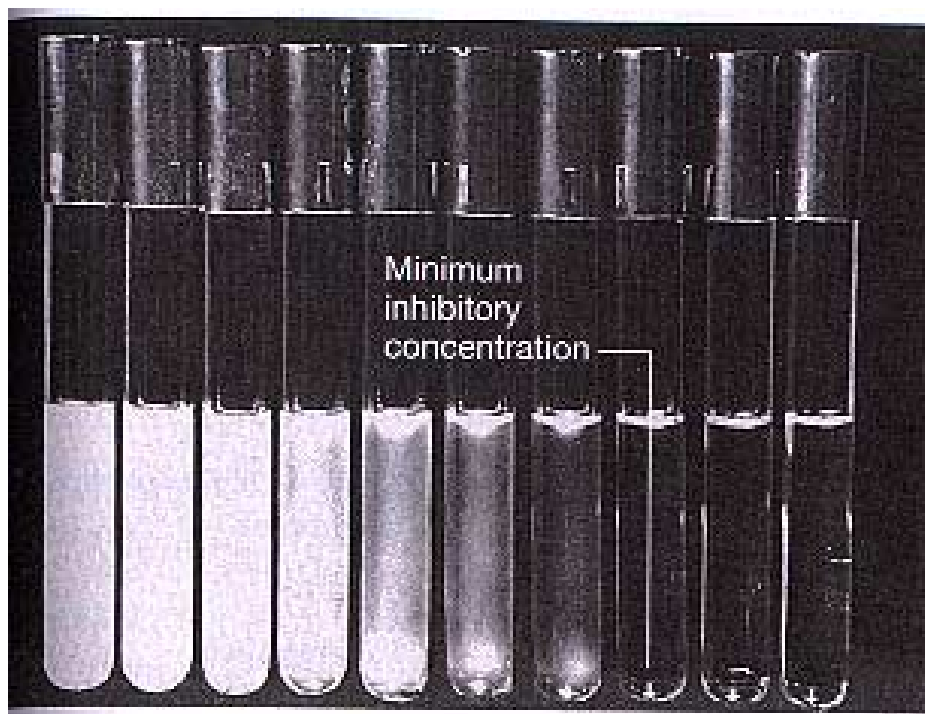
3 types of action of antimicrobial agents. At the time indicated by the arrow, a growth-inhibitory concentration of the agent was added to the exponentially growing culture. Note that the bacteriostatic agent inhibits growth without killing cells, bacteriocidal and bacteriolytic agents kill. Cell # decline when lysed.

## Many factors influence the effectiveness of an antimicrobial agent:

- microbial population size
- microbial population and community composition
- microbial encasement within surface biofilms
- concentration of the antimicrobial agent; may be “-static” at certain concentrations and “-cidal” at higher concentrations
- duration of exposure of the microorganisms to antimicrobial agent
- temperature of treatment
- presence of other organic materials
- level of microbial resistance to the antimicrobial agent
- other characteristics of the local environment.

## Various ways to measure antimicrobial activity:

1. Tube dilution assay to detect the minimal inhibitory concentration (MIC). A series of increasing concentrations of the antimicrobial agent are prepared in the culture broth medium. Each tube is equally inoculated and incubated to allow microbial growth to proceed. Growth (turbidity) occurs in those tubes containing the antimicrobial at concentrations below the MIC. Tube cultures are non-turbid (clear, no growth) at MIC and higher concentrations of the antimicrobial agent.



2. **Phenol coefficient test:** standardizes the potency of bacteriocidal agents in comparison with phenol using specific test microorganisms.

1. A series of dilutions of phenol and the experimental antimicrobial agent are prepared in sterile tubes.
2. Viable test bacteria (*Staphylococcus aureus* and *Salmonella typhi*) are inoculated into each tube and incubated at 37 °C.
3. After 10 minutes, samples of inoculated tubes are transferred into fresh culture medium without disinfectant and incubated for several more days.
4. The broth culture tubes are evaluated for growth by turbidity.
5. The highest dilutions of the test antimicrobial agent and of phenol that effectively kill the test organisms within 10 minute exposure are used to compute the phenol coefficient as follows:

$$\frac{1 / [\text{Effective dilution of test antimicrobial agent}]}{1 / [\text{Effective dilution of phenol}]}$$

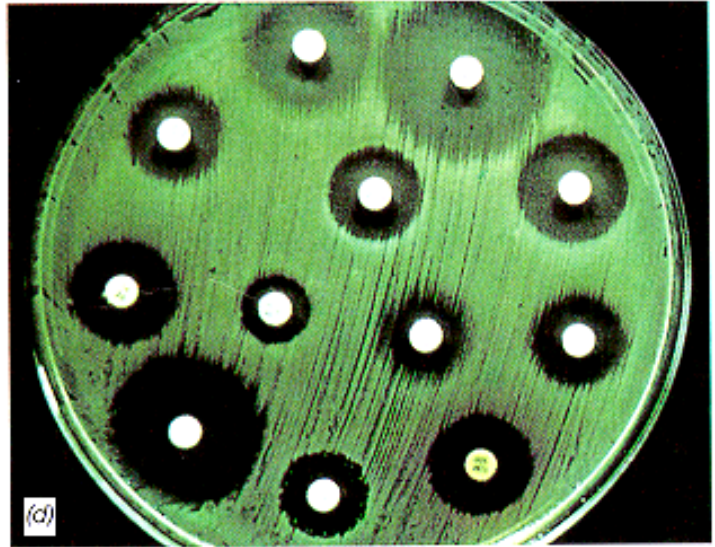
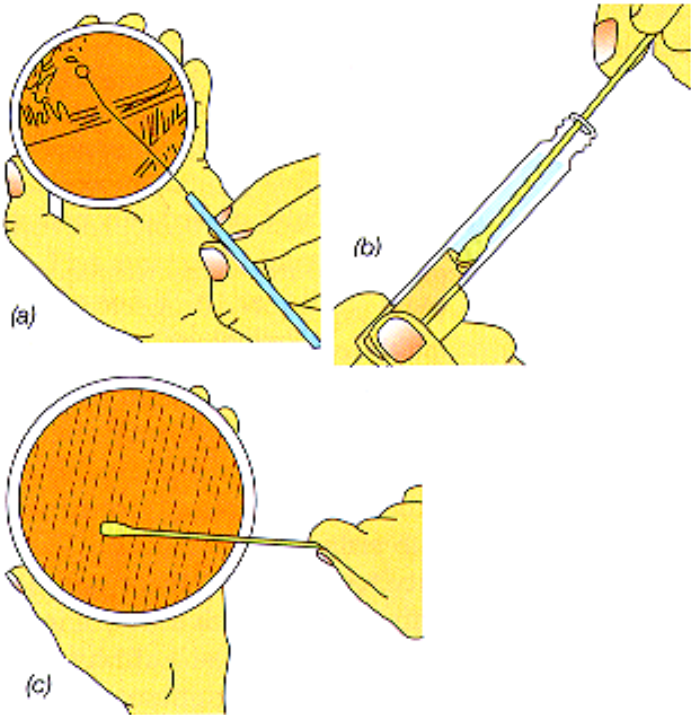
**Example:** If the highest effective dilution of the test antimicrobial = 1/500 and that of phenol = 1/100, the phenol coefficient of the test antimicrobial =  $500/100 = \underline{5}$ .

Agents with a phenol coefficient > 1 are more effective than phenol.

The higher the phenol coefficient value, the more effective the test antimicrobial is in killing bacteria under the test conditions.

Note that phenol coefficient tests are only valid for bacteriocidal (not bacteriostatic) antimicrobials, since cells inhibited (but not killed) by the latter would resume growth when transferred to fresh medium.

3. **Antibiotic disc agar diffusion** method: The test organism is spread on the culture medium in an agar plate and then sterile antibiotic discs are applied. After incubation, the organism produces a confluent “lawn” of growth except in **zones of inhibition** around discs containing antibiotics to which the organism is susceptible.



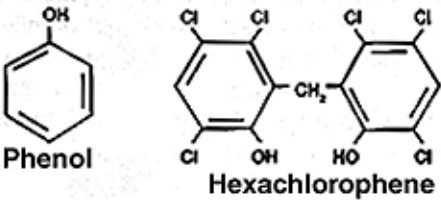
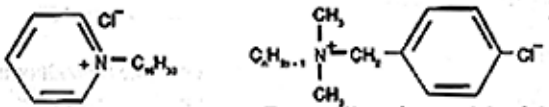
**Agar diffusion** of antibiotics producing zones of inhibition around discs on plate cultures containing a confluent lawn of growth.

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**Non-chemotherapeutic antimicrobials: antiseptics and disinfectants:**  
(too toxic for internal use)

- **Antiseptics** are antimicrobial agents that are sufficiently nontoxic to be useful in decontaminating the surface of living tissues.
- **Disinfectants** (also called “germicides”) are antimicrobial agents that are too toxic even for topical use on human tissue but can be used to decontaminate or sterilize nonliving materials.

## Examples of Antiseptics, disinfectants, and sterilants

Agent	Use	Mode of action
<b>Antiseptics</b>		
Alcohol (60–85% ethanol or isopropanol in water) <sup>a</sup>	Skin	Lipid solvent and protein denaturant
Phenol-containing compounds (hexachlorophene, triclosan, chloroxylenol, chlorhexidine)	Soaps, lotions, cosmetics, body deodorants	Disrupts cell membrane
	$\text{CH}_3\text{—CH}_2\text{—OH}$ Ethanol $\text{CH}_3\text{—CH(OH)—CH}_3$ Isopropanol  Phenol Hexachlorophene	
Cationic detergents, especially quaternary ammonium compounds (benzalkonium chloride)	Soaps, lotion	Interact with phospholipids of membrane
	 Cetylpyridinium chloride Benzalkonium chloride	
Hydrogen peroxide <sup>a</sup> (3% solution)	Skin	Oxidizing agent
Iodine-containing iodophor compounds in solution <sup>a</sup> (Betadine®)	Skin	Iodinates tyrosine residues of proteins; oxidizing agent
Organic mercury compounds <sup>b</sup> (mercurochrome)	Skin	Combines with -SH groups of proteins
Silver nitrate	Eyes of newborn to prevent blindness due to infection by <i>Neisseria gonorrhoeae</i>	Protein precipitant
<b>Disinfectants and sterilants</b>		
Chlorine gas	Disinfectant for purification of water supplies	Oxidizing agent
Chlorine compounds (chloramines, sodium hypochlorite, chlorine dioxide)	Disinfectant for dairy and food industry equipment, and water supplies	Oxidizing agent
Copper sulfate	Algicide in swimming pools, water supplies (disinfectant)	Protein precipitant
Ethylene oxide (gas)	Sterilant for temperature-sensitive laboratory materials such as plastics	Alkylating agent
Formaldehyde	3%–8% solution used as surface disinfectant, 37% (formalin) or vapor used as sterilant	Alkylating agent
Ozone	Disinfectant for drinking water	Strong oxidizing agent
Phenolic compounds <sup>b</sup>	Disinfectant for laboratory surfaces	Protein denaturant

<sup>a</sup> Alcohols, hydrogen peroxide, and iodine-containing iodophor compounds can act as antiseptics, disinfectants, or even sterilants depending on concentration, length of exposure, and form of delivery.

<sup>b</sup> Heavy metal (mercury) compounds and phenolic compounds, especially when used at high concentrations and in high volumes, produce environmentally hazardous waste products.

- Major use of disinfectants in the water purification industry: use chlorine to kill human fecal pathogens in the potable water supply
- Disinfectants are commonly used in many industrial applications, which can lead to toxic waste problems. Examples:
  1. Paper-pulp industry: organic mercurials & phenols are used to prevent microbial growth during manufacture
  2. Textile industry uses heavy metals & phenols to prevent microbial deterioration of fabrics exposed to the outside environment
  3. Wood industry uses phenols to prevent deterioration of wood in the outside environment
  4. Petroleum industry uses mercurials, phenols, & cationic detergents to prevent bacterial growth during recovery and storage of petroleum and petroleum products
  5. Electrical power industry uses chlorine to prevent bacterial growth in condensers and cooling towers
  6. Nuclear power industry uses chlorine in nuclear reactors to prevent growth of radiation-resistant bacteria (*Deinococcus radiodurans*).

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**Chemotherapeutic Antimicrobials:** compounds that exhibit sufficient selective toxicity toward microbes so that they can be taken internally for the control of infectious diseases.

Measure selective toxicity as the Therapeutic Index, calculated as:

Toxic dose (level at which the compound becomes toxic to host)

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Therapeutic dose (level required for effective chemical treatment)

The higher the Therapeutic Index, the better the chemotherapeutic agent. May have side effects (undesirable consequences to the host).

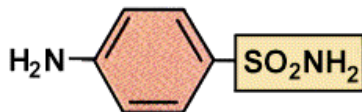
## Two major categories of chemotherapeutic antimicrobials:

- **Synthetic:** not made by microbes, often are chemical analogs that inhibit necessary step(s) in microbial metabolic pathways
- **Antibiotic:** made by certain microbes when they end exponential phase and enter stationary phase, will inhibit other microbes at low concentrations

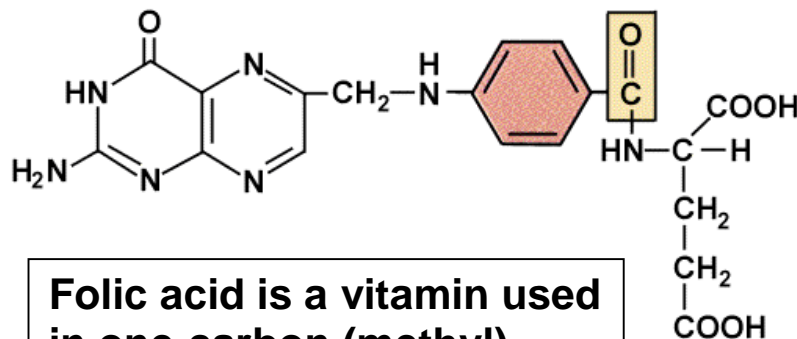
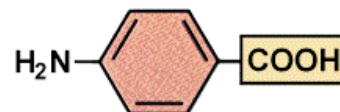
### Example of a synthetic chemotherapeutic antimicrobial:

The sulfa drug, sulfanilamide, is an analog of p-aminobenzoic acid, and inhibits the biosynthetic pathway that uses p-ABA as an intermediate in the synthesis of the vitamin, folic acid. Bacteria synthesize folic acid, humans don't → we must obtain it in our diet.

Sulfanilamide



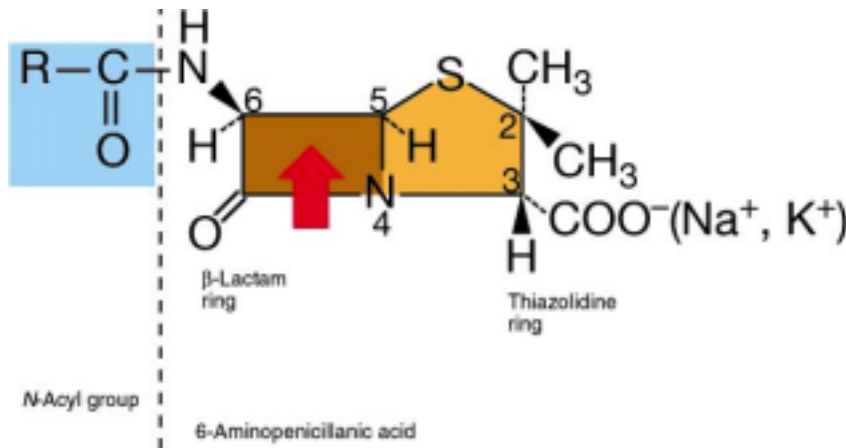
p-aminobenzoic acid



Folic acid is a vitamin used in one-carbon (methyl) transfer metabolism, e.g., purine biosynthesis.

## Example of a chemotherapeutic antibiotic:

Penicillin “ $\beta$ -lactam” antibiotics. Inhibit bacterial cell wall synthesis; specifically the peptide crosslinking of adjacent peptidoglycan chains needed to form the rigid murein sacculus. →weakened wall → protoplast lyses, kills the bug.



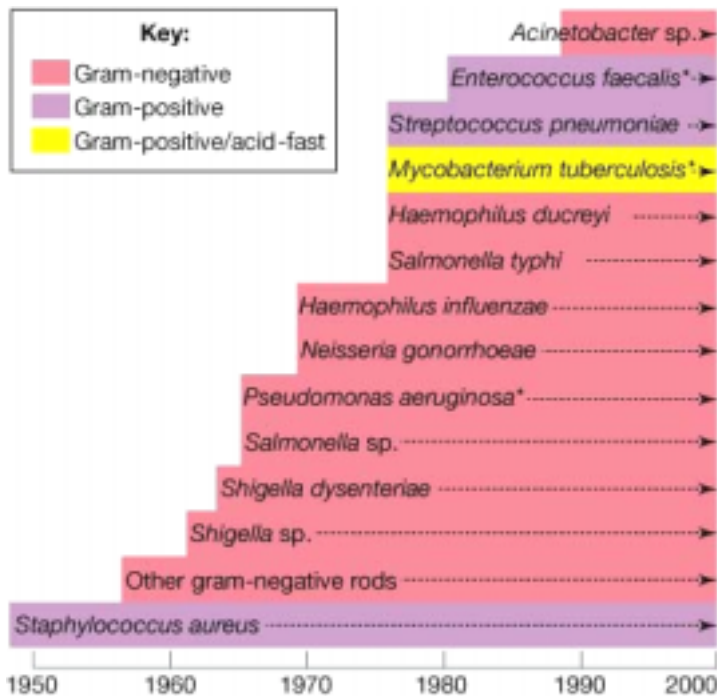
## Other classes of effective antibiotics:

- Cephalosporins – inhibit bacterial cell wall synthesis
- aminoglycosides – inhibit bacterial protein synthesis
- microlides – “
- tetracyclines - “

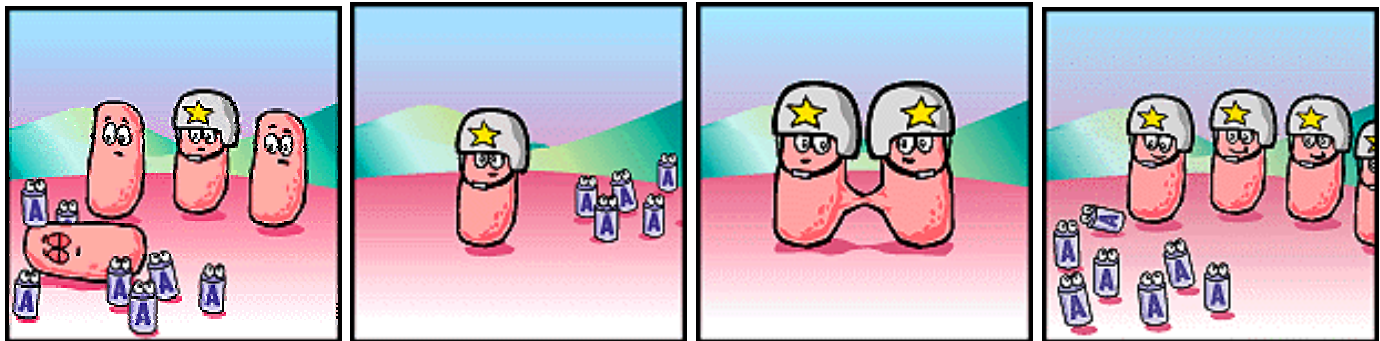
The continued search, screening, and manufacture of new antibiotics is a major component of the microbiologically-based biotechnology/pharmaceutical industry.



## Serious health care problem of antibiotic-resistant microbes:



Antibiotic-resistance is an inherited trait based on genes commonly encoded on self-transmissible resistance plasmids (R-plasmids) that efficiently spread from one microbe to another, particularly when exposed to the selective pressure of antibiotics in the environment (e.g., hospital).



## Mechanisms of microbial resistance to chemotherapeutic antimicrobials:

- Enzymes chemically modify and inactivate the antibiotic directly, e.g.,  $\beta$ -lactamases cleave the  $\beta$ -lactam ring in penicillins
- The microbe is impermeable to the antimicrobial.
- Bacterial mutation alters the target (e.g., ribosome component) so it no longer interacts with the antimicrobial.
- Bacterial mutation results in a metabolic “bypass” around the step specifically inhibited by the antimicrobial.
- The microbe uses an “efflux pump” to actively pump out the antimicrobial drug after it is taken up.