The Control of Microbial Growth

Introduction

The control of microbial growth is necessary in many practical situations, and significant advances in agriculture, medicine, and food science have been made through study of this area of microbiology.

"Control of growth", as used here, means to prevent growth of microorganisms. This control is effected in two basic ways:

- By killing microorganisms or
- By inhibiting the growth of microorganisms.

Control of growth usually involves the use of physical or chemical agents which either kill or prevent the growth of microorganisms. Agents which kill cells are called **cidal** agents; agents which inhibit the growth of cells (without killing them) are referred to as **static** agents. Thus the term **bactericidal** refers to killing bacteria and **bacteriostatic** refers to inhibiting the growth of bacterial cells. A **bactericide** kills bacteria, a **fungicide** kills fungi, and so on.

Sterilization is the complete destruction or elimination of all viable organisms (in or on an object being sterilized). There are no degrees of sterilization: an object is either sterile or not. Sterilization procedures involve the use of heat, radiation or chemicals, or physical removal of cells.

Methods of Sterilization

Heat: most important and widely used. For sterilization always consider type of heat, time of application and temperature to ensure destruction of all microorganisms. Endospores of bacteria are considered the most thermoduric of all cells so their destruction guarantees sterility.

1. **Incineration**: burns organisms and physically destroys them. Used for needles , inoculating wires, glassware, etc. and objects not destroyed in the incineration process.
2. **Boiling**: 100°C for 30 minutes. Kills everything except some endospores (Actually, for the purposes of purifying drinking water 100°C for five minutes is probably adequate though there have been some reports that Giardia cysts can survive this process). To kill endospores, and therefore **sterilize** the solution, very long or **intermittent boiling** is required.
3. **Autoclaving (steam under pressure or pressure cooker)**: 121°C for 15 minutes (15#/in^2 pressure). Good for sterilizing almost anything, but heat-labile substances will be denatured or destroyed.

4. **Dry heat (hot air oven)**: 160°C/2hours or 170°C/1hour. Used for glassware, metal, and objects that won't melt.

The protocol and recommendations for the use of heat to control microbial growth are given in Table 1.

Table 1. Recommended use of heat to control bacterial growth

Treatment	Temperature	Effectiveness
Incineration	>500°C	Vaporizes organic material on nonflammable surfaces but may destroy many substances in the process
Boiling	100°C	30 minutes of boiling kills microbial pathogens and vegetative forms of bacteria but may not kill bacterial endospores
Intermittent boiling	100°C	Three 30-minute intervals of boiling, followed by periods of cooling kills bacterial endospores
Autoclave and pressure cooker (steam under pressure)	121°C/15 minutes at 15# pressure	kills all forms of life including bacterial endospores. The substance being sterilized must be maintained at the effective T for the full time
Dry heat (hot air oven)	160°C/2 hours	For materials that must remain dry and which are not destroyed at T between 121°C and 170°C Good for glassware, metal, not plastic or rubber items
Dry heat (hot air oven)	170°C/1 hour	Same as above. Note increasing T by 10 degrees shortens the sterilizing time by 50 percent
Pasteurization (batch method)	63°C/30 minutes	kills most vegetative bacterial cells including pathogens such as streptococci, staphylococci and *Mycobacterium tuberculosis*
Pasteurization (flash method)	72°C/15 seconds	Effect on bacterial cells similar to batch method; for milk, this method is more conducive to industry and has fewer undesirable effects on quality or taste

Irradiation: usually destroys or distorts nucleic acids. Ultraviolet light is usually used (commonly used to sterilize the surfaces of objects), although x-rays and microwaves are possibly useful.

Filtration: involves the physical removal (exclusion) of all cells in a liquid or gas, especially important to sterilize solutions which would be denatured by heat (e.g. antibiotics, injectable drugs, amino acids, vitamins, etc.)

Chemical and gas: (formaldehyde, glutaraldehyde, ethylene oxide) toxic chemicals kill all forms of life in a specialized gas chamber.

Physical Agents

The lethal **temperature** varies in microorganisms. The **time** required to kill depends on the number of organisms, species, nature of the product being heated, pH, and temperature. Whenever heat is used to control microbial growth inevitably **both time and temperature are considered**.

1. **Sterilization** (boiling, autoclaving, hot air oven) kills all microorganisms with heat; commonly employed in canning, bottling, and other sterile packaging procedures.
2. **Pasteurization** is the use of mild heat to reduce the number of microorganisms in a product or food. In the case of pasteurization of milk the time and temperature depend on killing potential pathogens that are transmitted in milk, i.e., staphylococci, streptococci, *Brucella abortus* and *Mycobacterium tuberculosis*. For pasteurzation of milk: batch method: 63°C for 30 minutes; flash method: 71 °C for 15 seconds.

Low temperature (refrigeration and freezing): Most organisms grow very little or not at all at 0°C. Store perishable foods at low temperatues to slow rate of growth and consequent spoilage (e.g. milk). Low temperatures are not bactericidal. Psychrotrophs, rather than true psychrophiles, are the usual cause of food spoilage in refrigerated foods. *Lysteria moncytogenes* is of great concern in refrigerated foods and has been the topic of recent news articles and FDA action.

Drying (removal of H_2O): Most microorganisms cannot grow at reduced water activity (Aw < 0.90). Often used to preserve foods (e.g. fruits, grains, etc.). Methods involve removal of water from product by heat, evaporation, freeze-drying, addition of salt or sugar.

Irradiation (microwave, UV, x-ray): destroys microorganisms as described under sterilization. Many spoilage organisms are easily killed by irradiation. In some parts of Europe, fruits and vegetables are irradiated to increase their shelf life up to 500 percent. The practice has not been accepted in the U.S. Although the FDA has approved its use for meat. Public attitudes are starting to change with the recent food borne outbreaks and positive articles about irradiation from reputable sources.

Controlling Growth with Chemical Agents

Antimicrobial agents are chemicals that kill or inhibit the growth microorganisms. Antimicrobial agents include chemical preservatives and antiseptics, as well as drugs used in the treatment of infectious diseases of plants and animals. Antimicrobial agents may be of natural or synthetic origin, and they may have a static or cidal effect on microorganisms.

Types of antimicrobial agents

Antiseptics: microbicidal agents harmless enough to be applied to the skin and mucous membrane; should not be taken internaslly. Examples: mercurials, silver nitrate, iodine solution, alcohols, detergents.

Disinfectants: Agents that kill microorganisms, but not necessarily their spores,not safe for application to living tissues; they are used on inanimate objects such as tables, floors, utensils, etc. Examples: chlorine, hypochlorites, chlorine compounds, lye, copper sulfate, quaternary ammonium compounds.

Note: disinfectants and antiseptics are distinguished on the basis of whether they are safe for application to mucous membranes. Often, safety depends on the concentration of the compound. For example, sodium hypochlorite (chlorine), as added to water is safe for drinking, but "chlorox" , an excellent disinfectant, is hardly safe to drink.

Common antiseptics and disinfectants and their uses are summarized in Table 2.

Table 2. Common antiseptics and disinfectants

Chemical	Action	Uses
Ethanol (50-70%)	Denatures proteins and solubilizes lipids	Antiseptic used on skin
Isopropanol (50-70%)	Denatures proteins and solubilizes lipids	Antiseptic used on skin
Formaldehyde (8%)	Reacts with NH2, SH and COOH groups	Disinfectant, kills endospores

Tincture of Iodine (2% I2 in 70% alcohol)	Inactivates proteins	Antiseptic used on skin
Chlorine (Cl2) gas	Forms hypochlorous acid (HClO), a strong oxidizing agent	Disinfect drinking water; general disinfectant
Silver nitrate (AgNO3)	Precipitates proteins	General antiseptic and used in the eyes of newborns
Mercuric chloride	Inactivates proteins by reacting with sulfide groups	Disinfectant, although occasionally used as an antiseptic on skin
Detergents (e.g. quaternary ammonium compounds)	Disrupts cell membranes	Skin antiseptics and disinfectants
Phenolic compounds(e.g. carboloic acid, lysol, hexylresorcinol, hexachlorophene)	Denature proteins and disrupt cell membranes	Antiseptics at low concentrations; disinfectants at high concentrations
Ethylene oxide gas	Alkylating agent	Disinfectant used to sterilize heat-sensitive objects such as rubber and plastics

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Preservatives: static agents used to inhibit the growth of microorganisms, most often in foods. If eaten they should be nontoxic. Examples; calcium propionate, sodium benzoate, formaldehyde, nitrate, sulfur dioxide. Table 3 is a list of common preservative and their uses.

Table 3. Common food preservatives and their uses

Preservative	Effective Concentration	Uses
Propionic acid and propionates	0.32%	Antifungal agent in breads, cake, Swiss cheeses
Sorbic acid and sorbates	0.2%	Antifungal agent in cheeses, jellies, syrups, cakes
Benzoic acid and benzoates	0.1%	Antifungal agent in margarine, cider, relishes, soft drinks

Sodium diacetate	0.32%	Antifungal agent in breads
Lactic acid	?	Antimicrobial agent in cheeses, buttermilk, yogurt and pickled foods
Sulfur dioxide, sulfites	200-300 ppm	Antimicrobial agent in dried fruits, grapes, molasses
Sodium nitrite	200 ppm	Antibacterial agent in cured meats, fish
Sodium chloride	?	Prevents microbial spoilage of meats, fish, etc.
Sugar	?	Prevents microbial spoilage of preserves, jams, syrups, jellies, etc.
Wood smoke	NA	Prevents microbial spoilage of meats, fish, etc.

Antibiotics

Chemotherapeutic agents: antimicrobial agents of synthetic origin useful in the treatment of microbial or viral disease. Examples: sulfonilamides, isoniazid, ethambutol, AZT, chloramphenicol. Note that the microbiologist's definition of a chemotherapeutic agent requires that the agent be used for antimicrobial purposes and so excludes synthetic agents used for therapy against diseases that are not of microbial origin.

Antibiotics: antimicrobial agents produced by microorganisms that kill or inhibit other microorganisms. This is the microbiologist's definition. A more broadened definition of an antibiotic includes any chemical of natural origin (from any type of cell) which has the effect to kill or inhibit the growth of other types cells. Since most clinically-useful antibiotics are produced by microorganisms and are used to kill or inhibit infectious Bacteria, we will follow the classic definition.

Antibiotics are low molecular-weight (non-protein) molecules produced as secondary metabolites, mainly by microorganisms that live in the soil. Most of these microorganisms form some type of a spore or other dormant cell, and there is thought to be some relationship (besides temporal) between antibiotic production and the processes of sporulation. Among the molds, the notable antibiotic producers are Penicillium and Cephalosporium , which are the main source of the beta-lactam antibiotics (penicillin and its relatives). In the Bacteria, the Actinomycetes,

notably *Streptomyces* species, produce a variety of types of antibiotics including the aminoglycosides (e.g. streptomycin), macrolides (e.g. erythromycin), and the tetracyclines. Endospore-forming Bacillus species produce polypeptide antibiotics such as polymyxin and bacitracin. The table below (Table 4) is a summary of the classes of antibiotics and their properties including their biological sources.

Table 4. Classes of antibiotics and their properties

Chemical class	Examples	Biological source	Spectrum (effective against)	Mode of action
Beta-lactams (penicillins and cephalosporins)	Penicillin G, Cephalothin	*Penicillium notatum* and *Cephalosporium* species	Gram-positive bacteria	Inhibits steps in cell wall (peptidoglycan) synthesis and murein assembly
Semisynthetic penicillin	Ampicillin, Amoxycillin		Gram-positive and Gram-negative bacteria	Inhibits steps in cell wall (peptidoglycan) synthesis and murein assembly
Clavulanic Acid	Clavamox is clavulanic acid plus amoxycillin	*Streptomyces clavuligerus*	Gram-positive and Gram-negative bacteria	Suicide inhibitor of beta-lactamases
Monobactams	Aztreonam	*Chromobacter violaceum*	Gram-positive and Gram-negative bacteria	Inhibits steps in cell wall (peptidoglycan) synthesis and murein assembly
Carboxypenems	Imipenem	*Streptomyces cattleya*	Gram-positive and Gram-negative bacteria	Inhibits steps in cell wall (peptidoglycan) synthesis and murein assembly

Aminoglycosides	Streptomycin	*Streptomyces griseus*	Gram-positive and Gram-negative bacteria	Inhibit translation (protein synthesis)
	Gentamicin	*Micromonospora* species	Gram-positive and Gram-negative bacteria esp. Pseudomonas	Inhibit translation (protein synthesis)
Glycopeptides	Vancomycin	*Streptomyces orientales*	Gram-positive bacteria, esp. Staphylococcus aureus	Inhibits steps in murein (peptidoglycan) biosynthesis and assembly
Lincomycins	Clindamycin	*Streptomyces lincolnensis*	Gram-positive and Gram-negative bacteria esp. anaerobic Bacteroides	Inhibits translation (protein synthesis)
Macrolides	Erythromycin	*Streptomyces erythreus*	Gram-positive bacteria, Gram-negative bacteria not enterics, Neisseria,Legionella, Mycoplasma	Inhibits translation (protein synthesis)
Polypeptides	Polymyxin	*Bacillus polymyxa*	Gram-negative bacteria	Damages cytoplasmic membranes
	Bacitracin	*Bacillus subtilis*	Gram-positive bacteria	Inhibits steps in murein (peptidoglycan) biosynthesis and assembly
Polyenes	Amphotericin	*Streptomyces nodosus*	Fungi	Inactivate membranes containing sterols

	Nystatin	*Streptomyces noursei*	Fungi (Candida)	Inactivate membranes containing sterols
Rifamycins	Rifampicin	*Streptomyces mediterranei*	Gram-positive and Gram-negative bacteria, Mycobacterium tuberculosis	Inhibits transcription (eubacterial RNA polymerase)
Tetracyclines	Tetracycline	*Streptomyces*species	Gram-positive and Gram-negative bacteria, Rickettsias	Inhibit translation (protein synthesis)
Semisynthetic tetracycline	Doxycycline		Gram-positive and Gram-negative bacteria, Rickettsias Ehrlichia, Borellia	Inhibit translation (protein synthesis)
Chloramphenicol	Chloramphenicol	*Streptomyces venezuelae*	Gram-positive and Gram-negative bacteria	Inhibits translation (protein synthesis)

Antimicrobial Agents Used in the Treatment of Infectious Disease

The modern era of antimicrobial chemotherapy began in 1929 with Fleming's discovery of the powerful bactericidal substance penicillin, and Domagk's discovery in 1935 of synthetic chemicals (sulfonamides) with broad antimicrobial activity. In the early 1940's, spurred partially by the need for antibacterial agents in WW II, penicillin was isolated, purified and injected into experimental animals, where it was found to not only cure infections but also to possess incredibly low toxicity for the animals. This fact ushered into being the age of antibiotic chemotherapy and an intense search for similar antimicrobial agents of low toxicity to animals that might prove useful in the treatment of infectious disease. The rapid isolation of streptomycin, chloramphenicol and tetracycline soon followed, and by the 1950's, these and several other antibiotics were in clinical usage.

The most important property of a clinically-useful antimicrobial agent, especially from the patient's point of view, is its **selective toxicity**, i.e., that the agent acts in some way that inhibits or kills bacterial pathogens but has little or no toxic effect on the

animal taking the drug This implies that the biochemical processes in the bacteria are in some way different from those in the animal cells, and that the advantage of this difference can be taken in chemotherapy. Antibiotics may have a cidal (killing) effect or a static (inhibitory) effect on a range of microbes. The range of bacteria or other microorganisms that are affected by a certain antibiotic are is expressed as its **spectrum of action**. Antibiotics effective against procaryotes which kill or inhibit a wide range of Gram-positive and Gram-negative bacteria are said to be **broad spectrum** . If effective mainly against Gram-positive or Gram-negative bacteria, they are **narrow spectrum** . If effective against a single organism or disease, they are referred to as **limited spectrum**.

Kinds of Antimicrobial Agents and their Primary Modes of Action

1. **Cell wall synthesis inhibitors** Cell wall synthesis inhibitors generally inhibit some step in the synthesis of bacterial peptidoglycan. Generally they exert their selective toxicity against eubacteria because human cells lack cell walls.

   Beta lactam antibiotics Chemically, these antibiotics contain a 4-membered beta lactam ring. They are the products of two groups of fungi, Penicillium and Cephalosporium molds, and are correspondingly represented by the penicillins and cephalosporins. The beta lactam antibiotics inhibit the last step in peptidoglycan synthesis, the final cross-linking between between peptide side chains, mediated by bacterial carboxypeptidase and transpeptidase enzymes . Beta lactam antibiotics are normally bactericidal and require that cells be actively growing in order to exert their toxicity.

   Natural penicillins, such as **Penicillin G** or **Penicillin V**, are produced by fermentation of Penicillium chrysogenum. They are effective against streptococcus, gonococcus and staphylococcus, except where resistance has developed. They are considered narrow spectrum since they are not effective against Gram-negative rods.

   Semisynthetic penicillins first appeared in 1959. A mold produces the main part oif the molecule (6-aminopenicillanic acid) which can be modified chemically by the addition of side shains. Many of these compounds have been developed to have distinct benefits or advantages over penicillin G, such as increased spectrum of activity (effectiveness against Gram-negative rods), resistance to penicillinase, effectiveness when administered orally, etc. **Amoxycillin** and **Ampicillin** have broadened spectra against Gram-negatives and are effective orally; **Methicillin** is penicillinase-resistant.

   Clavulanic acid is a chemical sometimes added to a semisynthetic penicillin preparation. Thus, **amoxycillin** plus **clavulanate is clavamox** or **augmentin**. The clavulanate is not an antimicrobial agent. It inhibits beta lactamase enzymes and has given extended life to penicillinase-sensitive beta lactams.

   Although nontoxic, penicillins occasionally cause death when administered to persons who are allergic to them. In the U.S. there are 300 - 500 deaths annually due to penicillin

allergy. In allergic individuals the beta lactam molecule attaches to a serum protein which initiates an IgE-mediated inflammatory response.

Cephalolsporins are beta lactam antibiotics with a similar mode of action to penicillins that are produced by species of Cephalosporium. The have a low toxicity and a somewhat broader spectrum than natural penicillins. They are often used as penicillin substitutes, against Gram-negative bacteria, and in surgical prophylaxis. They are subject to degradation by some bacterial beta-lactamases, but they tend to be resistant to beta-lactamases from S. aureus .

Bacitracin is a polypeptide antibiotic produced by Bacillus species. It prevents cell wall growth by inhibiting the release of the muropeptide subunits of peptidoglycan from the lipid carrier molecule that carries the subunit to the outside of the membrane Teichoic acid synthesis, which requires the same carrier, is also inhibited. Bacitracin has a high toxicity which precludes its systemic use. It is present in many topical antibiotic preparations, and since it is not absorbed by the gut, it is given to "sterilize" the bowel prior to surgery.

2. **Cell membrane inhibitors** disorganize the structure or inhibit the function of bacterial membranes. The integrity of the cytoplasmic and outer membranes is vital to bacteria, and compounds that disorganize the membranes rapidly kill the cells. However, due to the similarities in phospholipids in eubacterial and eukaryotic membranes, this action is rarely specific enough to permit these compounds to be used systemically. The only antibacterial antibiotic of clinical importance that acts by this mechanism is **Polymyxin**, produced by Bacillus polymyxis. Polymyxin is effective mainly against Gram-negative bacteria and is usually limited to topical usage. Polymyxins bind to membrane phospholipids and thereby interfere with membrane function. Polymyxin is occasionally given for urinary tract infections caused by Pseudomonas that are gentamicin, carbenicillin and tobramycin resistant. The balance between effectiveness and damage to the kidney and other organs is dangerously close, and the drug should only be given under close supervision in the hospital.
3. **Protein synthesis inhibitors** Many therapeutically useful antibiotics owe their action to inhibition of some step in the complex process of translation. Their attack is always at one of the events occurring on the ribosome and rather than the stage of amino acid activation or attachment to a particular tRNA. Most have an affinity or specificity for 70S (as opposed to 80S) ribosomes, and they achieve their selective toxicity in this manner. The most important antibiotics with this mode of action are the **tetracyclines**,**chloramphenicol**, the **macrolides** (e.g. erythromycin) and the aminoglycosides (e.g. streptomycin).

   The **aminoglycosides** are products of *Streptomyces* species and are represented by streptomycin, kanamycin, tobramycin and gentamicin. These antibiotics exert their activity by binding to bacterial ribosomes and preventing the initiation of protein synthesis. Aminoglycosides have been used against a wide variety of bacterial infections caused by Gram-positive and Gram-negative bacteria. **Streptomycin** has been used extensively as a primary drug in the treatment of tuberculosis. **Gentamicin** is active

against many strains of Gram-positive and Gram-negative bacteria, including some strains of Pseudomonas aeruginosa. **Kanamycin** (a complex of three antibiotics, A, B and C) is active at low concentrations against many Gram-positive bacteria, including penicillin-resistant staphylococci. Gentamicin and **Tobramycin** are mainstays for treatment of Pseudomonas infections. An unfortunate side effect of aminoglycosides has tended to restrict their usage: prolonged use is known to impair kidney function and cause damage to the auditory nerves leading to deafness.

The **tetracyclines** consist of eight related antibiotics which are all natural products of *Streptomyces*, although some can now be produced semisynthetically. **Tetracycline**, **chlortetracycline** and **doxycycline** are the best known. The tetracyclines are broad-spectrum antibiotics with a wide range of activity against both Gram-positive and Gram-negative bacteria. The tetracyclines act by blocking the binding of aminoacyl tRNA to the A site on the ribosome. Tetracyclines inhibit protein synthesis on isolated 70S or 80S (eukaryotic) ribosomes, and in both cases, their effect is on the small ribosomal subunit. However, most bacteria possess an active transport system for tetracycline that will allow intracellular accumulation of the antibiotic at concentrations 50 times as great as that in the medium. This greatly enhances its antibacterial effectiveness and accounts for its specificity of action, since an effective concentration cannot be accumulated in animal cells. Thus a blood level of tetracycline which is harmless to animal tissues can halt protein synthesis in invading bacteria.

The tetracyclines have a remarkably low toxicity and minimal side effects when taken by animals. The combination of their broad spectrum and low toxicity has led to their overuse and misuse by the medical community and the wide-spread development of resistance has reduced their effectiveness. Nonetheless, tetracyclines still have some important uses, such as in the treatment of Lyme disease.

Chloramphenicol has a broad spectrum of activity but it exerts a bacteriostatic effect. It is effective against intracellular parasites such as the rickettsiae. Unfortunately, aplastic anemia, which is dose related develops in a small proportion (1/50,000) of patients. Chloramphenicol was originally discovered and purified from the fermentation of a *Streptomyces*, but currently it is produced entirely by chemical synthesis. Chloramphenicol inhibits the bacterial enzyme peptidyl transferase thereby preventing the growth of the polypeptide chain during protein synthesis.

Chloramphenicol is entirely selective for 70S ribosomes and does not affect 80S ribosomes. Its unfortunate toxicity towards the small proportion of patients who receive it is in no way related to its effect on bacterial protein synthesis. However, since mitochondria probably originated from procaryotic cells and have 70S ribosomes, they are subject to inhibition by some of the protein synthesis inhibitors including chloroamphenicol. This likely explains the toxicity of chloramphenicol. The eukaryotic cells most likely to be inhibited by chloramphenicol are those undergoing rapid multiplication, thereby rapidly synthesizing mitochondria. Such cells include the blood forming cells of the bone marrow, the inhibition of which could present as aplastic anemia. Chloramphenicol was once a highly prescribed antibiotic and a number of deaths

from anemia occurred before its use was curtailed. Now it is seldom used in human medicine except in life-threatening situations (e.g. typhoid fever).

The **Macrolides** are a family of antibiotics whose structures contain large lactone rings linked through glycoside bonds with amino sugars. The most important members of the group are **erythromycin** and **oleandomycin**. Erythromycin is active against most Gram-positive bacteria, Neisseria, Legionella and Haemophilus, but not against the Enterobacteriaceae. Macrolides inhibit bacterial protein synthesis by binding to the 50S ribosomal subunit. Binding inhibits elongation of the protein by peptidyl transferase or prevents translocation of the ribosome or both. Macrolides are bacteriostatic for most bacteria but are cidal for a few Gram-positive bacteria.

4. **Effects on Nucleic Acids** Some chemotherapeutic agents affect the synthesis of DNA or RNA, or can bind to DNA or RNA so that their messages cannot be read. Either case, of course, can block the growth of cells. The majority of these drugs are unselective, however, and affect animal cells and bacterial cells alike and therefore have no therapeutic application. Two nucleic acid synthesis inhibitors which have selective activity against procaryotes and some medical utility are nalidixic acid and rifamycins.

   Nalidixic acid is a synthetic chemotherapeutic agent which has activity mainly against Gram-negative bacteria. Nalidixic acid belongs to a group of compounds called **quinolones**. Nalidixic acid is a bactericidal agent that binds to the DNA gyrase enzyme (topoisomerase) which is essential for DNA replication and allows supercoils to be relaxed and reformed. Binding of the drug inhibits DNA gyrase activity.

   Some quinolones penetrate macrophages and neutrophils better than most antibiotics and are thus useful in treatment of infections caused by intracellular parasites. However, the main use of nalidixic acid is in treatment of lower urinary tract infections (UTI). The compound is unusual in that it is effective against several types of Gram-negative bacteria such as E. coli, Enterobacter aerogenes, K. pneumoniae and Proteus species which are common causes of UTI. It is not usually effective against Pseudomonas aeruginosa, and Gram-positive bacteria are resistant.

   The **rifamycins** are also the products of *Streptomyces*. **Rifampicin** is a semisynthetic derivative of rifamycin that is active against Gram-positive bacteria (including Mycobacterium tuberculosis) and some Gram-negative bacteria. Rifampicin acts quite specifically on eubacterial RNA polymerase and is inactive towards RNA polymerase from animal cells or towards DNA polymerase. The antibiotic binds to the beta subunit of the polymerase and apparently blocks the entry of the first nucleotide which is necessary to activate the polymerase, thereby blocking mRNA synthesis. It has been found to have greater bactericidal effect against M .tuberculosis than other anti-tuberculosis drugs, and it has largely replaced isoniazid as one of the front-line drugs used to treat the disease, especially when isoniazid resistance is indicated. It is effective orally and penetrates well into the cerebrospinal fluid and is therefore useful for treatment of tuberculosis meningitis and meningitis caused by Neisseria meningitidis.

5. **Competitive Inhibitors** The competitive inhibitors are mostly all synthetic chemotherapeutic agents. Most are "growth factor analogs" which are structurally similar to a bacterial growth factor but which do not fulfill its metabolic function in the cell. Some are bacteriostatic and some are bactericidal.

   Sulfonamides were introduced as chemotherapeutic agents by Domagk in 1935, who showed that one of these compounds (prontosil) had the effect of curing mice with infections caused by beta-hemolytic streptococci. Chemical modifications of the compound sulfanilamide gave compounds with even higher and broader antibacterial activity. The resulting sulfonamides have broadly similar antibacterial activity, but differ widely in their pharmacological actions. Bacteria which are almost always sensitive to the sulfonamides include Streptococcus pneumoniae, beta-hemolytic streptococci and E. coli. The sulfonamides have been extremely useful in the treatment of uncomplicated UTI caused by E. coli, and in the treatment of meningococcal meningitis (because they cross the blood-brain barrier).

   The sulfonamides (e.g. **Gantrisin**) and **Trimethoprim** are inhibitors of the bacterial enzymes required for the synthesis of tetrahydofolic acid (THF), the vitamin form of folic acid essential for 1-carbon transfer reactions. Sulfonamides are structurally similar to para aminobenzoic acid (PABA), the substrate for the first enzyme in the THF pathway, and they competitively inhibit that step . Trimethoprim is structurally similar to dihydrofolate (DHF) and competitively inhibits the second step in THF synthesis mediated by the DHF reductase. Animal cells do not synthesize their own folic acid but obtain it in a preformed fashion as a vitamin. Since animals do not make folic acid, they are not affected by these drugs, which achieve their selective toxicity for bacteria on this basis.

   Three additional synthetic chemotherapeutic agents have been used in the treatment of tuberculosis: **isoniazid (INH)**, **paraaminosalicylic acid (PAS)**, and **ethambutol**. The usual strategy in the treatment of tuberculosis has been to administer a single antibiotic (historically streptomycin, but now, most commonly, rifampicin is given) in conjunction with INH and ethambutol. Since the tubercle bacillus rapidly develops resistance to the antibiotic, ethambutol and INH are given to prevent outgrowth of a resistant strain. It must also be pointed out that the tubercle bacillus rapidly develops resistance to ethambutol and INH if either drug is used alone. Ethambutol inhibits incorporation of mycolic acids into the mycobacterial cell wall. Isoniazid has been reported to inhibit mycolic acid synthesis in mycobacteria and since it is an analog of pyridoxine (Vitamin B6) it may inhibit pyridoxine catalyzed reactions as well. Isoniazid is activated by a mycobacterial peroxidase enzyme and destroys several targets in the cell. PAS is an anti-folate. PAS was once a primary anti-tuberculosis drug, but now it is a secondary agent, having been largely replaced by ethambutol.