Microbial Control

By Imran Ali, PhD

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Chemical control

- Chemical agents are generally not intended to achieve sterilization.
- Most reduce the microbial populations to safe levels or remove pathogens from objects.
- An ideal disinfectant or antiseptic (chemical agent) kills microorganisms in the shortest possible time without damaging the material treated.
- Among the important criteria for selecting an antiseptic or disinfectant are the concentration of disinfectant to be used, whether the agent is bactericidal or bacteriostatic, the nature of the material to be treated, whether organic matter will be present, the temperature and pH at which the chemical agent will be used, and the time available in which the chemical agent will be left in contact with the surface tested.

- **Evaluation methods:** To evaluate an antiseptic or disinfectant, the **phenol coefficient test** is used.
- In this test, various dilutions of the chemical agent are prepared and tested against equivalent dilutions of phenol with such bacteria as *Staphylococcus aureus* and *Salmonella typhi*.
- A phenol coefficient (PC) greater than one indicates that the chemical agent is more effective than phenol and less than one that it is less effective.
- An alternative test is the **in-use test.** Various dilutions of the chemical agent are made and tested against a standardized preparation of test bacteria on the type of material later to be disinfected in normal use.

- **Phenol:** One of the first chemicals to be used for disinfection was **phenol.**
- First used by Joseph Lister in the 1860s, it is the standard for most other antiseptics and disinfectants.
- Phenol derivatives called **phenolics** contain altered molecules of phenol useful as antiseptics and disinfectants.
- The phenolics damage cell membranes and inactivate enzymes of microorganisms, while denaturing their proteins.
- They include **cresols**, such as Lysol, as well as several **bisphenols**, such as hexachlorophene, which is particularly effective against staphylococci.
- A chemical agent resembling the phenols is **chlorhexidine** (Hibiclens), which is used for skin disinfection as an alternative to hexachlorophene. It persists on the skin and is effective against vegetating bacteria, but not spores.

- **Halogens.** Among the **halogen** antiseptics and disinfectants are chlorine and iodine.**Iodine** is used as a tincture of iodine, an alcohol solution. Combinations of iodine and organic molecules are called **iodophors**. They include Betadine and Isodyne, both of which contain a surface active agent called povidone. Iodine combines with microbial proteins and inhibits their function.
- **Chlorine** also combines with microbial proteins. It is used as sodium hypochlorite (bleach). As calcium hypochlorite, chlorine is available to disinfect equipment in dairies, slaughterhouses, and restaurants. The chloramines contain chlorine together with ammonia. They are used to sanitize glassware and eating utensils and are effective in the presence of organic matter. Chlorine is also used as a gas to maintain a low microbial count in drinking water.

- **Alcohols. Alcohols** are useful chemical agents when employed against bacteria and fungi, but they have no effect on bacterial spores. The type of alcohol most widely used is 70 percent **ethyl alcohol** (ethanol). **Isopropyl alcohol** (rubbing alcohol) is also useful as an antiseptic and disinfectant. Because alcohols evaporate quickly, they leave no residue and are useful in degerming the skin before injections.
- **Heavy metals.** A number of **heavy metals** have antimicrobial ability. For example,**silver** is used as silver nitrate in the eyes of newborns to guard against infection by*Neisseria gonorrheae*. It is also used to cauterize wounds. **Copper** is used as copper sulfate to retard the growth of algae in swimming pools, fish tanks, and reservoirs. **Zinc**is useful as zinc chloride in mouthwashes and as zinc oxide as an antifungal agent in paints. The heavy metals are believed to act by combining with sulfhydryl groups on cellular proteins.

- **Soaps and detergents.** Soaps and detergents decrease the surface tension between microorganisms and surfaces, and thereby help cleanse the surface. **Soaps** emulsify the oily film on the body surface, carrying the oils, debris, and microorganisms away in a degerming action. The cationic **detergents** are **quaternary ammonium compounds.**They solubilize the cell membranes of microorganisms. Among the popular compounds are Zephiran (benzalkonium chloride) and Cepacol (cetylpyridinium chloride).
- **Aldehydes.** Two **aldehydes**, formaldehyde and glutaraldehyde, inactivate microbial proteins by crosslinking the functional groups in the proteins. **Formaldehyde** gas is commonly used as formalin, a 37 percent solution of formaldehyde gas. It is widely employed for embalming purposes. **Glutaraldehyde** is used as a liquid to sterilize hospital equipment. However, several hours are required to destroy bacterial spores

- **Ethylene oxide.** Sterilization can be achieved with a chemical known as **ethylene oxide (ETO).** This chemical denatures proteins and destroys all microorganisms, including bacterial spores. It is used at warm temperatures in an ethylene oxide chamber. Several hours are needed for exposure and flushing out the gas, which can be toxic to humans. ETO is widely used for plastic instruments such as Petri dishes, syringes, and artificial heart valves. **Propylene oxide**, a similar compound, is also valuable as a sterilant.
- **Oxidizing agents.** Oxidizing agents such as **hydrogen peroxide** kill microorganisms by releasing large amounts of oxygen, which contributes to the alteration of microbial enzymes. Hydrogen peroxide is useful on inanimate objects and in foods, but on the skin surface, it is quickly broken down by the enzyme catalase, liberating oxygen. This oxygen causes a wound to bubble and thereby removes microorganisms present. However, the chemical activity on the skin is limited compared to that on inanimate surfaces. Contact lenses can be disinfected with hydrogen peroxide.

- Two other oxidizing agents are **benzoyl peroxide** and **ozone.** Benzoyl peroxide is applied to the skin to treat acne due to anaerobic bacteria. The oxygen released by the compound inhibits anaerobic growth. Ozone can be used to disinfect water, where it oxidizes the cellular components of contaminating microbes.
- **Food preservatives.** Foods can be preserved by using a number of **organic acids** to maintain a low microbial population. Sorbic acid is used in a number of acidic foods, including cheese, to prevent microbial growth. Benzoic acid also inhibits fungi and is used in acidic foods and soft drinks. Calcium propionic acid prevents the growth of mold in breads and bakery products.

Antibiotics

- Various families of antibiotics are used for various types of microorganisms to achieve control and assist body defenses during times of infection.
- **Antibiotics** are products of microorganisms that react with and inhibit the growth of other microorganisms.
- An antibiotic should be selectively toxic to pathogenic microorganisms, should not incite an allergic response in the body, should not upset the normal microbial population of various body sites, and should not foster the development of drug resistance.

- **Penicillin** prevents Gram-positive bacteria from forming peptidoglycan, the major component of the cell wall.
- Without peptidoglycan, internal pressures cause the bacterium to swell and burst.
- Penicillin is not one antibiotic, but a family of antibiotics.
- The family includes penicillin F, penicillin G, and penicillin X, as well as ampicillin, amoxicillin, nafcillin, and ticarcillin.
- The first penicillin was derived from the green mold *Penicillium*, but most penicillins are now produced by synthetic means.
- A few are used against Gram-negative bacteria.
- People allergic to penicillin may suffer localized allergy reactions or whole body reactions known as anaphylaxis.
- Long-term use of penicillin encourages the emergence of penicillin-resistant bacteria because these bacteria produce penicillinase, an enzyme that converts penicillin to penicilloic acid.

- **Cephalosporin antibiotics** include cefazolin, cefoxitin, cefotaxime, cefuroxime, and moxalactam.
- The antibiotics were first produced by the mold *Cephalosporium*.
- They prevent synthesis of bacterial cell walls, and most are useful against Gram-positive bacteria; the newer cephalosporin antibiotics are also effective against Gram-negative bacteria.
- Cephalosporins are especially useful against penicillin-resistant bacteria and are often used as substitutes for penicillin.
- The **aminoglycoside antibiotics** inhibit protein synthesis in Gram-negative bacteria.
- Members of this antibiotic group include gentamicin, kanamycin, tobramycin, and streptomycin.
- Originally isolated from members of the actinomycetes genus : *Streptomyces*, the aminoglycosides are now produced synthetically or semisynthetically.
- Streptomycin is effective against the tuberculosis bacterium. Unfortunately, many aminoglycosides have a deleterious effect on the ear and impair hearing.

- **Tetracycline antibiotics** are broad spectrum drugs that inhibit the growth of Gram-negative bacteria, rickettsiae, chlamydiae, and certain Gram-positive bacteria.
- They accomplish this by inhibiting protein synthesis.
- Compared to other antibiotics, tetracyclines have relatively mild side effects, but they are known to destroy helpful bacteria in the body.
- Also, they interfere with calcium deposit in the body, so they should not be used in very young children.
- Originally isolated from members of the genus *Streptomyces*, the tetracyclines include such antibiotics as minocycline, doxycycline, and tetracycline.

- **Other antibacterial antibiotics:** The antibiotic **erythromycin,** may be used as a substitute for penicillin when penicillin sensitivity or penicillin allergy exists.
- Erythromycin is useful against Gram-positive bacteria and has been found effective against the organisms that cause Legionnaires' disease and mycoplasmal pneumonia. It inhibits protein synthesis.
- Tuberculosis is a difficult disease to treat because the etiologic agent is the extremely resistant bacterium *Mycobacterium tuberculosis*.
- Five drugs are currently useful for treating tuberculosis: **rifampin, ethambutol, streptomycin, para-aminosalicylic acid**, and **isoniazid.**
- Rifampin is also used to treat bacterial meningitis.

- **Bacitracin** is used for the treatment of skin infections due to Gram-positive bacteria.
- This antibiotic inhibits cell wall synthesis in bacteria and can be used internally, but it may cause kidney damage.
- **Vancomycin** is currently used against bacteria displaying resistance to penicillin, cephalosporin, and other antibiotics.
- Vancomycin is a very expensive antibiotic with numerous side effects, and it is used only in life-threatening situations.
- It interferes with cell wall formation in bacteria.

- **Chloramphenicol** is effective against a broad range of bacteria including Gram-positive and Gram-negative bacteria, rickettsiae, and chlamydiae.
- However, it has serious side effects such as aplastic anemia (blood cells without hemoglobin), and it may induce the gray syndrome (a type of cardiovascular collapse) in babies.
- Therefore, it is used for only the most serious bacterial infections such as typhoid fever and meningitis.
- Sulfa drugs such as **sulfamethoxazole** and **sulfisoxazole** are effective against Gram-positive bacteria.
- These bacteria produce folic acid, and the sulfa drugs interfere with its production by replacing para-aminobenzoic acid (PABA) in the folic acid molecule.
- This action typifies how an antibiotic can interfere with a metabolic pathway in bacteria.

- **Antifungal drugs:** Several **antifungal antibiotics** are currently available for treating infectious disease.
- One example is **griseofulvin**, which is used against the fungi of ringworm and athlete's foot. Other examples are **nystatin, clotrimazole, ketoconazole,** and **miconazole**, all of which are used against infections due to *Candida albicans*.
- For systemic fungal infections, the antibiotic **amphotericin B** is available, although it has serious side effects.

- **Antiviral drugs:** viral drugs are not widely available because viruses have few functions or structures with which drugs can interfere.
- Nevertheless, certain drugs are available to interfere with viral replication.
- One example is **azidothymidine (AZT)**, which is used to interrupt the replication of human immunodeficiency virus.
- Other examples are **acyclovir**, which is used against herpes viruses and chickenpox viruses; **ganciclovir**, which is used against cytomega-lovirus; **amantadine**, which is prescribed against influenza viruses; and **interferon**, which has been used against rabies viruses and certain cancer viruses.
- **Antiprotozoal drugs.** Many antibiotics used against bacteria, for example, tetracycline, are also useful against protozoa.
- Among the drugs used widely as antiprotozoal agents are metronidazole (Flagyl), which is used against *Trichomonas vaginalis;* quinine, which is used against malaria; and pentamidine isethionate, which is valuable against *Pneumocystis carinii*.

- **Drug resistance:** Over the past decades, **drug-resistant** strains have developed in bacteria.
- These strains probably existed in the microbial population, but their resistance mechanisms were not needed because the organisms were not confronted with the antibiotic.
- With widespread antibiotic use, the susceptible bacteria died off, and the resistant bacteria emerged.
- They multiplied to form populations of drug-resistant microorganisms.
- Microorganisms can exhibit their resistance in various ways. For example, they can release enzymes (such as penicillinase) to inactivate the antibiotic before the antibiotic kills the microorganism; or they can stop producing the drug-sensitive structure or modify the structure so that it is no longer sensitive to the drug; or they can change the structure of the plasma membrane so that the antibiotic cannot pass to the cytoplasm.

Microbial growth control

- The control of microbial growth may involve sterilization, disinfection, antisepsis, sanitization, or degerming.
- Sterilization is the destruction of all forms of microbial life, with particular attention to bacterial spores.
- Disinfection and antisepsis both refer to destruction of microbial pathogens, although some organisms, such as bacterial spores, may remain alive.
- Disinfection refers to the destruction of pathogenic organisms on an inanimate (lifeless) object, such as a table-top, while antisepsis refers to that destruction on a living object, such as the skin surface.

- **Sanitization** refers to the reduction in the number of pathogens to a level deemed safe by public health guidelines.
- **Degerming** is the physical removal of microorganisms by using such things as soaps or detergents.
- Any chemical agent that kills microorganisms is known as a **germicide.**
- An agent that destroys bacteria is called a **bactericide**, one that kills fungi is a **fungicide**, and one that kills viruses is a **viricide.**
- **A bacteriostatic agent** prevents the further multiplication of bacteria without necessarily killing all that are present.

- Among the conditions affecting the use of a germicide are temperature, the type of microorganism, and the environment.
- Germicides are more effective at high temperatures because the chemical breaks down at lower temperatures.
- Microorganisms vary in their susceptibility depending on such things as the composition of their cell wall, the presence or absence of a capsule, and the ability to form spores or cysts.
- The environment can affect the activity of a germicide, as, for example, when organic matter is present. This material shields microorganisms from germicides and often reacts with the germicide.

Physical methods of control

- Physical methods for controlling the growth of microorganisms can be divided into heat methods and nonheat methods.
- The lowest temperature at which all microorganisms are killed in 10 minutes is the **thermal death point**, while the minimum amount of time required to kill microorganisms at a given temperature is known as the **thermal death time.**
- The time for destruction of 90 percent of the microbial population is the **decimal reduction time.**

- **Dry heat:** kills microorganisms by reacting with and oxidizing their proteins.
- Dry heat can be used in incineration devices, such as the **Bunsen burner** or the **hot-air oven.**
- In the hot-air oven, a temperature of about 170°C for two hours will bring about sterilization.
- **Moist heat:** is used to kill microorganisms in such things as **boiling water.**
- Most vegetating microorganisms are killed within two or three minutes, but over two or three hours may be required for destruction of bacterial spores.
- In moist heat, the microbial proteins undergo **denaturation**, a process in which the three-dimensional form of the protein reverts to a two-dimensional form, and the protein breaks down.

- Moist heat is used in the **autoclave**, a high-pressure device in which steam is superheated.
- Steam at 100°C is placed under a pressure of 15 pounds per square inch, increasing the temperature to 121°C.
- At this temperature, the time required to achieve sterilization is about 15 minutes.
- The autoclave is the standard instrument for preparing microbial media and for sterilizing instruments such as syringes, hospital garb, blankets, intravenous solutions, and myriad other items.

- Although **pasteurization** is used to lower the bacterial content of milk and dairy products, it does not achieve sterilization.
- The conditions of pasteurization are set up to eliminate the tuberculosis bacillus and the rickettsia that causes Q fever.
- Milk is pasteurized for 30 minutes at about 62°C or for 15 to 17 seconds at about 72°C.
- The first method is known as the **holding method**, the second method as the **flash method.**
- Dairy products can be pasteurized at 82°C for three seconds, a process known as **ultrapasteurization.**
- An alternative heating method is **tyndallization**, also called **intermittent sterilization.**
- Liquids and other items are subjected to free-flowing steam for 30 minutes on each of three successive days.
- During the first day, all vegetating microorganisms, except spores, are killed. In the overnight period, the spores germinate, and they are killed by the steam on the second day.
- The last few remaining spores germinate on the second evening and are killed on the third day.

- **Nonheat methods.** A number of **nonheat methods** are also available to control the growth and presence of microorganisms.
- Among these is **filtration**, a process in which a liquid or gas passes through a series of pores small enough to retain microorganisms.
- A vacuum can be created to help pull the liquid or gas through the filter. A filter is often used when heat-sensitive materials such as vaccines are to be sterilized.
- Filter materials can be of various types. For example, certain filters consist of **diatomaceous earth**, the skeletal remains of diatoms.
- **Membrane filters** composed of nitrocellulose can also be used. The effectiveness of the filter depends upon the pore size, which can be established to trap the microorganisms desired.
- For instance, if bacteria are to be removed, the pore size would be about 0.15 µm, while if viruses are to be removed, the pores size should be about 0.01 µm.

- **Drying** can be used to control the growth of microorganisms because when water is removed from cells, they shrivel and die.
- To dry foods, they are mixed with salt or sugar. Either draws water out of microbial cells by osmosis, and they quickly die.
- One method for achieving drying is **lyophilization**, a process in which liquids are quick-frozen and then subjected to evacuation, which dries the material.
- Salted meat and sugared fruits are preserved this way.
- **Cold temperatures** are used in the refrigerator to control microbial growth.
- At low temperatures, microbial metabolism slows considerably, and the reproductive rate is reduced.
- However, cold temperatures do not necessarily kill microorganisms. At freezing temperatures, ice crystals kill many microorganisms present.

- **Radiations** are also used to control microorganisms when food or other materials are subjected to gamma rays or X rays.
- The radiations change the chemical composition of microorganisms by forming ions in the organic materials of the cytoplasm.
- Highly reactive toxic radicals also form.
- Nonionizing radiations are typified by **ultraviolet light.**
- Ultraviolet light affects the nucleic acids of microorganisms, inducing adjacent thymine residues in DNA molecules to bind to one another forming dimers.
- This binding changes the character of the DNA, making it unable to function in protein synthesis. Cell death soon follows.
- Although **microwaves** are a form of radiation, their direct effect on microorganisms is minimal.
- Microwaves induce water molecules to vibrate at high rates, creating heat.
- The heat is the killing agent rather than the microwaves.