Shree H. N. Shukla College of Science,
(Affiliated to Saurashtra university)
Nr. Lalpari Lake, B/H Marketing Yard, Rajkot-360 003

F.Y. B.Sc. (Sem. II) (CBCS)

MICROBIOLOGY

[201]: BASICS OF BIOCHEMISTRY AND MICROBIAL CONTROL

UNIT 4

CONTROL OF MICROORGANISMS BY PHYSICAL AND CHEMICAL AGENTS

Prepared By

KRUPA BARAVADIYA

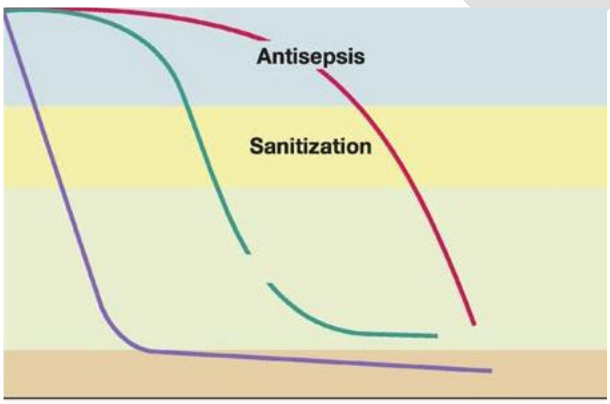
➢ INTRODUCTION

- the control and destruction of microorganisms, a topic of immense practical importance. Although most microorganisms are beneficial, some microbial activities have undesirable consequences, such as food spoilage and disease.
- Therefore it is essential to be able to kill a wide variety of microorganisms or inhibit their growth to minimize their destructive effects.
- The goal is twofold: (1) to destroy pathogens and prevent their transmission, and (2) to reduce or eliminate microorganisms responsible for the contamination of water, food, and other substances.
- Microorganism control continues to be a hot topic as microorganisms evolve to resist current strategies.
- Control efforts have a substantial role in public health to prevent disease as well as in therapeutic use to treat disease. Thus this chapter focuses on the control of microorganisms by physical, chemical, and biological agents (such as engineered bacteria).
- In general, any chemical, physical, or biological product that controls microorganisms is referred to as an antimicrobial agent.

▪ BASIC PRINCIPLE TO CONTROL THE MICROORGANISMS

- The principles of microbial control are rooted in microbial nutrition ,growth, and development.
- If you can starve or poison, or inhibit or prevent growth or replication, you can control microorganisms.
- Of course, it is not as simple as this sounds. Subtle differences in population, degree of killing, and even what is used to remove microorganisms result in a somewhat complex vocabulary.
- Terminology is especially important when the control of microorganisms is discussed because words such as disinfectant and antiseptic often are used loosely, and as we shall see, they have different meanings.
- The situation is even more confusing because a particular treatment can either inhibit growth or kill, depending on the conditions.
- The types of control agents and their uses are outlined. In general, to control microorganisms a biocide must be evaluated so as to determine the specific parameters under which it will be effective.
- **Sterilization** (Latin sterilis, unable to produce offspring or barren) is the process by which all living cells, spores, and acellular entities (e.g., viruses, viroids, and prions) are either destroyed
- or removed from an object or habitat.

- A sterile object is totally free of viable microorganisms, spores, and other infectious
- agents. When sterilization is achieved by a chemical agent, the chemical is called a sterilant.
- In contrast, disinfection is the killing, inhibition, or removal of microorganisms that may cause disease; disinfection is the substantial reduction of the
- total microbial population and the destruction of potential pathogens.
- **Disinfectants** are agents, usually chemical, used to
- carry out disinfection and normally used only on inanimate objects. A disinfectant does not necessarily sterilize an object because viable spores and a few microorganisms may remain.
- Sanitization is closely related to disinfection. In sanitization, the microbial population is reduced to levels that are considered safe by public health standards.
- The inanimate object is usually cleaned as well as partially disinfected. For example, **sanitizers** are used to clean eating utensils in restaurants.
- Viroids and satellites ; Prions It also is frequently necessary to control microorganisms on or in living tissue with chemical agents.
- **Antisepsis** (Greek anti, against, and sepsis, putrefaction) is the destruction or inhibition of microorganisms on living tissue; it is the prevention
- of infection or sepsis.
- Antiseptics are chemical agents applied to tissue to prevent infection by killing or inhibiting pathogen
- growth; they also reduce the total microbial population, Because they must not destroy too much host tissue, antiseptics are generally not as toxic as disinfectants.



- The exposure of microorganisms to increasing biocide concentrations decreases the number of viable organisms. figure shows three possible population reduction curves resulting from three different biocides.

- The shape of the curve reflects various conditions that influence biocide effectiveness. Note that in each case, the eventual decline in viable microorganisms can occur as a staged decline of viability from antisepsis to sterilization.
- **Chemotherapy** is the use of chemical agents to kill or inhibit the growth of microorganisms within host tissue.
- A suffix can be employed to denote the type of antimicrobial agent.
- Substances that kill organisms often have the suffix -cideLatin cida, to kill); a cidal agent kills pathogens (and many nonpathogens) but not necessarily endospores.
- A disinfectant or antiseptic can be particularly effective against a specific group, in which case it may be called a bactericide, fungicide, or viricide.
- Other chemicals do not kill but rather prevent growth. If these agents are removed, growth will resume. Their names end in -static (Greek statikos, causing to stand or stopping); for example, bacteriostatic and fungistatic.
- The Pattern of Microbial Death
- a lethal agent. Population death is generally exponential (logarithmic); that is, the population is reduced by the same fraction at constant intervals (table).
- If the logarithm of the population number remaining is plotted against the time of exposure of the microorganism to the agent, a straight-line plot will result
- (figure). When the population has been greatly reduced, the rate of killing may slow due to the survival of a more resistant strain of the microorganism.
- It is essential to have a precise measure of an agent's killing efficiency.
- One such measure is the decimal reduction time (D)
- or D value. The decimal reduction time is the time required to kill 90% of the microorganisms or spores in a sample under specified conditions.
- For example, in a semilogarithmic plot of th population remaining versus the time of heating, the D value is the time required for the line to drop by one log cycle or tenfold (figure a).
- It is also possible to determine the temperature change at a given D value that decreases the microbial population by one log cycle (90%). This temperature change is referred to as the Z value and is predicted from a semilogarithmic plot of D values versus temperature (figure b).
- To study the effectiveness of a lethal agent, one must be able to decide when microorganisms are dead, which may present some challenges. A microbial cell is often defined as dead if it does not grow when inoculated into culture medium that would normally support its growth. In like manner, an inactive virus cannot infect a suitable host.

- This definition has follaws, however. It has been demonstrated that when bacteria are exposed to certain conditions, they can remain alive but are temporarily unable to reproduce.

Pattern of microbial death

Microorganisms usually die logarithmically (i.e. the population will be reduced by the same fraction at regular intervals)

Table 7.1
A Theoretical Microbial Heat-Killing Experiment

Minute	Microbial Number at Start of Minute[a]	Microorganisms Killed in 1 Minute (90% of total)[a]	Microorganisms at End of 1 Minute	Log_{10} of Survivors
1	10^6	9×10^5	10^5	5
2	10^5	9×10^4	10^4	4
3	10^4	9×10^3	10^3	3
4	10^3	9×10^2	10^2	2
5	10^2	9×10^1	10	1
6	10^1	9	1	0
7	1	0.9	0.1	−1

[a]Assume that the initial sample contains 10^6 vegetative microorganisms per ml and that 90% of the organisms are killed during each minute of exposure. The temperature is 121° C.

- When in this state, these persister cells are often referred to as vviable but nonculturable (VBNC) In conventionalvtests to demonstrate killing by an antimicrobial agent VBNC bacteria would be thought to be dead.
- This is a serious problem because the bacteria may regain their ability to reproduce and cause infection after a period of recovery.

Sr.no.	Question	Answer
1	.... process are used to remove microorganisms fron an object	Sterilization
2	.... agent used to carry out disinfection.	Disinfectant
3	.... applied to tissue to prevent infections	Antisepsis
4	.... is used to kill microorganisms within host tissue	Chemotherapy
5	..........are used to clean eating utensils in restrorent	Senitizer

- **CHCRACTERISTICS EVALUTION AND SELECTION OF IDEAL ANTIMICROBIAL AGENTS**
- **1.Population size**. Because an equal fraction of a microbial population is killed during each interval, a larger population requires a longer time to die than does a small.
- **2. Population composition**. The effectiveness of an agent
- varies greatly with the nature of the organisms being treated because microorganisms differ markedly in susceptibility. Bacterial spores are much more resistant to most antimicrobial agents than are vegetative forms, and younger cells are usually more readily destroyed than
- mature organisms. Some species are able to withstand adverse conditions better than others. For instance, *M. tuberculosis*, which causes tuberculosis, is much more resistant to antimicrobial agents than most other bacteria.
- **3. Concentration or intensity of an antimicrobial agent.**
- Often, but not always, the more concentrated a chemical agent or intense a physical agent, the more rapidly microorganisms are destroyed. However, agent effectiveness usually is not directly related to concentration or intensity. Over a short range, a small increase in
- concentration leads to an exponential rise in effectiveness; beyond a certain point, increases may not raise the killing rate much at all. Sometimes an agent is more effective at lower concentrations. For example, 70% ethanol is more bacteriocidal than 95% ethanol because the activity of ethanol is enhanced by the presence of water.
- **4. Contact time.** The longer a population is exposed to a microbicidal agent, the more organisms are killed. To achieve sterilization, contact time should be long enough to reduce the probability of survival
- by at least 6 logs.
- **5. Temperature**. An increase in the temperature at which a chemical acts often enhances its activity. Frequently a lower concentration of disinfectant or sterilizing agent can be used at a higher temperature.
- **6. Local environment**. The population to be controlled is not isolated but surrounded by environmental factors that may either offer protection or aid in its destruction. For example, because heat kills more readily at an acidic pH, acidic foods and beverages such as fruits and tomatoes are easier to pasteurize than more alkaline foods such as milk.
- A second important environmental factor is organic and chemical disinfecting agents. Biofilms are a good example. The organic matter in a biofilm protects the biofilm's microorganisms. Furthermore, it has been clearly documented that bacteria in biofilms are altered physiologically, and

this makes them less susceptible to many antimicrobial agents. Because of the impact of organic matter, it may be necessary to clean objects, especially medical and dental equipment, before they are disinfected or sterilized.

Sr.no.	Question	Answer
1	.... is more resistant bacterial species which can cause TB	M. tuberculosis
2	The activity of ethanol is enhanced by	water
3	Sterilization agent can be used at .... temperature	Higher
4	For the sterilization process the contect time of organism should be......... log phase	6
5	.... in a biofilm protects the biofilm's microorganisms	The organic matter

MODE OF ACTION OF ANTIMICROBIAL AGENTS

- There are a number of mechanisms by which antimicrobial agent can injure or damage microbial cells. It is necessary to know how all agents kill or inhibit microorganisms. When microorganisms are exposed to different agents many changes are observed in their cellular processes. Therefore it is very difficult to determine the initial damage caused to cell brought about by all agent. The possible site of action of an antimicrobial agent ca be determined on the basis of certain features of microbial cell.
- **normal living organism contains:**
- A multitude of enzymes responsible for metal biology - OF are not cytoplasmic cytoplasmic membrane) me that controls the inflow and outflow of nutrient and metabolite between call and its external environment and it is so the side of my reaction The cell wall that provides a protective covering to the cell in its participating in certain physiological process damage to any of these areas initiates a number of subsequent changes Sending to the death of the cell . or control results

- **1)Damage to cell wall**
- : physical and The lowering of surface tension of the medium in which microorganisms are suspended often injures the cell wall . Substance which reduces surface tension are called surfactants . Soaps , bile al Gran man depends Cationic and anionic detergents are surfactants . The damage is

caused at least in part , by the dissolving or emulsifying effects of the surfactants on denaturation lipids . Several hydrolytic enzyme present in natural sources of some microorganisms , hydrolysate the microbial cell wall . The enzyme lysozyme found in tears , leucocyte , mucous secretion etc hydrolyses the cell wall of many bacteria . Antibiotics such as penicillin , cycloserine etc. inhibit the formation of cell wall material in a growing bacterial culture which results in the formation of protoplasts . Partial or complete removal of cell wall leads to hich is not the osmotic rupturing of the cell membrane and dissolves the cell (lysis)

- **2)Damage to the Cytoplasmic membrane**
- : Thus the Surfactants which dissolve lipids , disrupt the cytoplasmic membrane . This (destroys the selective permeability of nutrients and metabolites . This results in the disruption of normal metabolic processes , leading to inhibition of the face mat growth or death of the cell . The antimicrobial activity of phenolic compounds , ween the synthetic detergents soaps , quaternary ammonium compounds , antibiotics (polymyxin) etc. is due to their effect on the cell membrane,

- **. 3) Denaturation and Coagulation of protein**
- : For the normal metabolic activity of cell , its native structural configuration is necessary . Any substance or condition which affects or alters the native state of protein results in its denaturation or coagulation , causing damage to cell or may result in its killing 0.g. of such agent high temperature alcohol , formaldehyde etc. of the D)

- 4)**Inhibition of metabolic reactions** : d more Metabolic activity of a cell depends on various enzyme catalyzed reaction . An agent which inhibits any of these reactions therefore alters the normal metabolic pattern of a cell . Inhibition of energy generating pathways is particularly detrimental e.g. Glycolysis . Tri - carboxylic acid cycle , biological oxidation , oxidative phosphorylation are some of such pathways . Certain agents like fluoride , cyanide , trivalent arsenic compounds are e.g. of such inhibitors.

Sr.no.	Question	Answer
1	.... process damage the cell wall	Lowering of surface tension
2	Substance which reduced surface tension are	surfactents

	caleed...........	
3	.... enzymes is found in tears	Lysozymes
4	.... antibiotic inhibit the formation of cell wall	Penicillin
5	Give a name of inhibitor which inhibite biological pathway	Cyanide

- **SELECTION OF IDEAL ANTIMICROBIAL AGENTS**
- There is no single antimicrobial agent) process which can be successfully used The everywhere . The major factors that have to be assessed while selecting the most appropriate agent for specific practical application
- (1) **Nature of material to be treated** : The agent selected should be compatible earth with material on which it has to be applied environ.
- (2) **Type of microorganisms** : All agents are not equally effective against bacteria , fungi , viruses and other microorganisms . Spores are more resistant than vegetative cells . Therefore the agent selected must be known to the Effective against the type of the organism to be destroyed.
- 3) **Environmental condition:** temperature, pH, time, concentration and presence of extraneous organic material may have a bearing on the rate and efficiency of antimicrobial action.

- **<u>Physical Control Methods</u>**
- Heat and other physical agents are normally used to control microbial
- growth and sterilize objects, as can be seen from the operation of the autoclave. The most frequently employed physical agents are heat and radiation.

- **Heat**
- Moist heat readily destroys viruses, bacteria, and fungi .
- Moist heat kills by degrading nucleic acids and denaturing enzymes and other essential proteins. It also disrupts cell membranes. Exposure to boiling water for 10 minutes is sufficient to destroy vegetative cells and eukaryotic spores.
- Unfortunately, the temperature of boiling water (100°C or 212°F at
- sea level) is not sufficient to destroy bacterial spores, which may survive hours of boiling. Therefore boiling can be used for disinfection of drinking water and objects not harmed by water, but boiling does not sterilize.

- To destroy bacterial endospores, moist heat sterilization must be carried out at temperatures above 100°C, and this requires the use of saturated steam under pressure.
- Steam sterilization is carried out with an autoclave a device somewhat like a fancy pressure cooker. The development of the autoclave by Charles
- Chamberland in 1884 tremendously stimulated the growth of microbiology as a science.
- Water is boiled to produce steam, which is released into the autoclave's chamber , The air initially present in the chamber is forced out until the chamber is filled with saturated steam and the outlets are closed.
- Hot, saturated steam continues to enter until the chamber reaches the desired temperature are not as reliable as techniques that kill bacterial spores. The Autoclave. (a) A modern, automatically controlled autoclave or

  sterilizer. (b) Longitudinal
- cross section of a typical autoclave showing some of its parts and the pathway of steam.
- Many heat-sensitive substances, such as milk, are treated with controlled
- heating at temperatures well below boiling, a process known as pasteurization in honor of its developer, Louis Pasteur. In the 1860s the French wine industry was plagued by the problem of wine spoilage,
- which made wine storage and shipping difficult.
- Pasteur examined spoiled wine under the microscope and detected microorganisms that looked like the bacteria responsible for lactic acid and acetic acid fermentations; these bacteria do not form spores. He then discovered that a brief heating at 55 to 60°C would destroy these microorganisms and preserve wine for long periods.
- In 1886 the German chemists V. H. Soxhlet and F. Soxhlet adapted the
- technique for preserving milk and reducing milk-transmissible diseases.
- Milk pasteurization was introduced in the United States in 1889., and pressure, usually 121c and 15 pounds of pressure. At this temperature, saturated steam destroys all vegetative cells and spores in a small volume of liquid within 10 to 12 minutes.
- Treatment is continued for at least 15 minutes to provide a margin of safety. Of course, larger containers of liquid such as flasks and carboys require much longer treatment times.
- Autoclaving must be carried out properly or the processed materials will not be sterile. If all air has not been flushed out of the chamber, it will not reach 121°C, even though it may reach a pressure of 15 pounds. The chamber should not be packed too tightly because the steam needs to circulate freely

and contact everything in the autoclave. Bacterial spores will be killed only if they are kept at 121°C for 10 to 12 minutes.

- When a large volume of liquid must be sterilized, an extended sterilization time is needed because it takes longer for the centre of the liquid to reach 121 °C; 5 liters of liquid may require about 70 minutes. In view of these potential difficulties, a biological indicator is often autoclaved along with other material.
- This indicator commonly consists of a culture tube containing a sterile ampule of medium and a paper strip covered with spores of *Geobacillus stearothermophilus*.
- After autoclaving, the ampule is aseptically broken and the culture incubated for several days. If the test bacterium does not grow in the
- medium, the sterilization run has been successful.
- Sometimes indicator tape or paper that changes color upon sufficient heating is autoclaved with a load of material.
- These approaches that indicate heating has occurred are convenient and save time but Milk, beer, and many other beverages are now pasteurized. Pasteurization does not sterilize a beverage, but it does kill any pathogens present and drastically slows spoilage by reducing the level of non pathogenic spoilage microorganisms
- Some materials cannot withstand the high temperature of the autoclave, and spore contamination precludes the use of other methods to sterilize them. For these materials, a process of intermittent sterilization, also known as tyndallization (for John Tyndall, the British physicist who used the technique to destroy heat-resistant microorganisms in dust) is used. The process also uses steam (30-60 minutes) to destroy vegetative bacteria.
- However, steam exposure is repeated for a total of three times with 23- to 24-hour incubations between steam exposures.
- The incubations permit remaining spores to germinate into heat-sensitive vegetative cells that are then destroyed upon subsequent steam exposures.
- Many objects are best sterilized in the absence of water by dry heat sterilization.
- Some items are sterilized by incineration. For instance, inoculating loops, which are used routinely in the laboratory, can be sterilized in a small, bench-top incinerator Other items are sterilized in an oven at 160 to 170°C for 2 to 3 hours.
- Microbial death results from the oxidation of cell constituents and denaturation of proteins. Dry air heat is less effective than moist heat. The spores of Clostridium botulinum, the cause of botulism, are killed in 5 minutes at 121 o c by moist heat but only after 2 hours at 160°C by dry heat.

However, dry heat has some definite advantages. It does not corrode glassware and metal.

- **Dry Heat Incineration**
- instruments as moist heat does, and it can be used to sterilize powders, oils, and similar items. Despite these advantages, dry heat sterilization is slow and not suitable for heat-sensitive materials such as many plastic and rubber items.

Radiation

- radiation consists of waves and particles that are of sufficient energy to dislodge electrons from atoms or molecules, producing chemically reactive free radicals. The free radicals react with nearby matter to weaken or destroy it.
- Ionizing radiation will destroy bacterial spores and all microbial cells; however, ionizing radiation is not always effective against viruses.
- Gamma radiation from a cobalt 60 source and accelerated electrons from high-voltage electricity are used in the cold sterilization of antibiotics, hormones, sutures, and plastic disposable supplies such as syringes. Gamma radiation and electron beams have also been used to sterilize and "pasteurize" meat and other foods.
- Irradiation can eliminate the threat of such pathogens as E. coli 0157:H7, which causes a life threatening intestinal disease; Staphylococcus aureus, which causes skin and blood infections, and readily colonizes medical
- devices used on patients; and *Campylobacter jejuni,* which contaminates poultry, causing human intestinal disease when undercooked meat is eaten.
- Based on the results of numerous studies, both the U.S. Food and Drug Administration and the World Health Organization have approved irradiated food and declared it safe for human consumption. Currently irradiation is being used to treat poultry, beef, pork, veal, lamb, fruits, vegetables and spices. Human diseases caused by bacteria (Controlling food spoilage) List the advantages and disadvantages of ultraviolet light and ionizing radiation as sterilizing agents.
- Provide a few examples of how each is used for this purpose. What is the correlation between radiation "energy" and the mechanisms of sterilization?
- Radiation room Chamber with radiation shield Ultraviolet (UV) radiation around 260 nm is quite lethal. UV radiation causes thymine-thymine dimerization of DNA, preventing replication and transcription.
- However, UV does not penetrate glass, dirt films, water, and other substances very effectively. Because of this disadvantage, UV radiation is

used as a sterilizing agent only in a few specific situations. UV lamps are sometimes placed on the ceilings of rooms or in biological safety cabinets to sterilize the air and any exposed surfaces.

- Because UV radiation burns the skin and damages eyes, the UV lamps are off when the areas are in use. Commercial UV units are available for water treatment. Pathogens and other microorganisms are destroyed when a thin layer of water is passed under the lamps.

Sr.no.	Question	Answer
1	The most frequently employed physical agents are ....&........	Heat & radiation
2	How moist heat killed microorganisms?	Moist heat kills by degrading nucleic acids and denaturing enzymes and other essential proteins
3	Steam sterilization is carried out by.........	Autoclave
4	.... food is safe for human consumption	Irradiate food
5	UV radiation caused.........	Thymine-thymine dimerization

- **<u>Chemical Control Agents</u>**
- Physical agents are generally used to sterilize objects. Chemicals, on the other hand, are more often employed in disinfection and antisepsis. The proper use of chemical agents is essential to laboratory and hospital safety.
- Chemicals also are employed to prevent microbial growth in food, and certain chemicals are used to treat infectious disease. The use of chemical agents for chemotherapy in humans is covered in chapter 9. Here we discuss chemical used outside the body.
- Many different chemicals are available for use as disinfectants, and each has its own advantages and disadvantages. Ideally the disinfectant must be effective against a wide variety of infectious agents (Gram-positive and Gram-negative bacteria, acid-fast bacteria, bacterial spores, fungi, and viruses) at low concentrations and in the presence of organic matter.

Although the chemical must be toxic for infectious agents, it should not be toxic to people or corrosive for common materials.

- In practice, this balance between effectiveness and low toxicity for animals is hard to achieve. Some chemicals are used despite their low effectiveness because they are relatively nontoxic. The ideal disinfectant
- should be stable upon storage, odorless or with a pleasan odor, and soluble in water and lipids for penetration into microorganisms have a low surface tension so that it can enter cracks in surfaces; and be relatively inexpensive.
- Other chemicals are used as antiseptics. Recall that antiseptics are less toxic to humans than disinfectants and as such may be less effective at killing all the microorganisms that disinfectants can kill. In general, antiseptics should reduce the number of pathogens on human tissue to prevent infection.
- Examples of antiseptics include hand sanitizers, silver threads woven into clothing, and dilute iodine solutions that can be sprayed onto wounds.
- Antiseptics are meant to be used in the absence of thorough cleaning
- with soap and water. One potentially serious problem is the overuse of antiseptics.
- For instance, resistance to the antibacterial agent triclosan (found in products such as deodorants, mouthwashes, soaps, cutting boards, and baby toys) has become a problem. For example, P. aeruginosa actively pumps the antiseptic out of the cell. There is now evidence that extensive use of triclosan also increases the frequency of bacterial resistance to antibiotics.
- Thus overuse of antiseptics can have unintended harmful consequences. The properties and uses of several groups of common disinfectants and antiseptics are surveyed next.

- **Phenolics**
- Phenol was the first widely used antiseptic and disinfectant. In 1867 Joseph Lister employed it to reduce the risk of infection during surgery. Today phenol and phenol derivatives (phenolics) such as cresols, xylenols, and orthophenylphenol are used as disinfectants in laboratories and hospitals.
- The commercial disinfectant Lysol is made of a mixture of phenolics. Phenolics act by denaturing proteins and disrupting cell membranes.
- They have some real advantages as disinfectants: phenolics are tuberculocidal, effective in the presence of organic material, and remain active on surfaces long after application. However, they have a disagreeable odor and can cause skin irritation. The newer phenolic, triclosan , is often used in hand sanitizers due to its effective blockage of bacterial fatty acid synthesis.

- **Alcohols**
- Alcohols are among the most widely used disinfectants and antiseptics.
- They are bactericidal and fungicidal but not sporicidal; some enveloped viruses are also destroyed. The two most popular alcohol germicides are ethanol and isopropanol, usually used in about 60 to 80% concentration.
- They act by denaturing proteins and possibly by dissolving membrane lipids. A 10- to IS-minute soaking is sufficient to disinfect small instruments.

Sr.no.	Question	Answer
1	.........Actively pimps antiseptic out of the cell	P. aeruginosa
2	Chemical agents are more employed in	disinfection and antisepsis
3	.... is first wildly used antiseptic	Phenol
4	.... are bactericidal and fungicidal but not sporicidal	Alcohols
5	Give name of two most popular alcohol.	Ethanol & isopropanol

- **Halogens**
- A halogen is any of the five elements (fluorine, chlorine, bromine, iodine, and astatine) in group VIlA of the periodic table. They exist as diatomic molecules in the free state and form saltlike compounds with sodium and most other metals.
- The halogens iodine and chlorine are important antimicrobial agents.
- Iodine is used as a skin antiseptic and kills by oxidizing cell constituents
- and iodinating cell proteins. At higher concentrations, it may even kill some spores.
- Iodine often is applied as tincture of iodine, 2% or more iodine in a water-ethanol solution of potassium iodide.
- Although it is an effective antiseptic, the skin may be damaged, a stain is
- left, and iodine allergies can result. Iodine can be complexed with an organic carrier to form an iodophor.
- Iodophors are water soluble, stable, and nonstaining, and release iodine slowly to minimize skin burns and irritation.

- They are used in hospitals for cleansing preoperative skin and in hospitals and laboratories for disinfecting. Some popular brands are Wescodyne for skin and laboratory disinfection, and Betadine for wounds. Chlorine is the usual disinfectant for municipal water supplies and swimming pools, and is employed in the dairy and food industries.
- It may be applied as chlorine gas (Cl2), sodium hypochlorite (bleach, Na OCl), or calcium hypochlorite [Ca(OCl)z], all of which yield hypochlorous acid (HOCl):
- $Cl_2 + H_2O \rightarrow HCl + HOCl$
- $NaOCl + H_2O \rightarrow NaOH + HOCl$
- $Ca(OCl)_2 + 2H_2O \rightarrow Ca(OH)_2 + 2HOCl$
- The result is oxidation of cellular materials and destruction of vegetative bacteria and fungi.
- Death of almost all microorganisms usually occurs within 30 minutes. One potential problem is that chlorine reacts with organic compounds to form carcinogenic trihalomethanes, which must be monitored in drinking
- water.
- Some wastewater treatment facilities recover the chlorine from the water prior to discharge to prevent the formation of trihalomethanes.
- Chlorine is also an excellent disinfectant for individual use because it is effective, inexpensive, and easy to employ. Small quantities of drinking water can be disinfected with halazone tablets.
- Halazone (parasulfone dichloramidobenzoic acid) slowly releases chloride when added to water and disinfects it in about a half hour. It is frequently used by campers lacking access to uncontaminated drinking water. Of note is the fact that household bleach (diluted to 10% in water, 10-minute contact time) can be used to disinfect surfaces contaminated by human
- body fluids and that it is made more effective by the addition of household vinegar.

- **Heavy Metals**
- For many years the ions of heavy metals such as mercury, silver arsenic, zinc, and copper were used as germicides. These have now been superseded by other less toxic and more effective germicides (many heavy metals are more bacteriostatic thanbactericidal). There are a few exceptions.
- In some hospitals, a 1% solution of silver nitrate is added to the eyes of infants to prevent ophthalmic gonorrhea. Silver sulfadiazine is used on burns.
- Copper sulfate is an effective algicide in lakes and swimming pools.
- Heavy metals combine with proteins, often with their sulfhydryl groups, and inactivate them. They may also precipitate cell proteins.

- **Quaternary Ammonium Compounds**
- Quaternary ammonium compounds are detergents that have broad spectrum antimicrobial activity and are effective disinfectants used for decontamination purposes.
- Detergents (Latin detergere, to wipe away) are organic cleansing agents that are amphipathic, having both polar hydrophilic and nonpolar hydrophobic components. The hydrophilic portion of a quaternary ammonium compound is a positively charged quaternary nitrogen;
- thus quaternary ammonium compounds are cationic detergents. Their antimicrobial activity is the result of their ability to disrupt microbial membranes; they may also denature proteins.
- Cationic detergents such as benzalkonium chloride and cetylpyridinium
- chloride kill most bacteria but not M. tuberculosis or spores. They have the advantages of being stable and nontoxic, but they are inactivated by hard water and soap.
- Cationic detergents are often used as disinfectants for food utensils and small instruments, and as skin antiseptics.

Sr.no.	Question	Answer
1	What is use of iodine?	Iodine is used as a skin antiseptic and kills by oxidizing cell constituents
2	Give name of cationic detergents	benzalkonium chloride and cetylpyridinium
3	.... is used as a usual disinfrct in municipal water supply	Chlorine
4	What are detergents?	Detergent are organic cleansing agents that are amphipathic, having both polar hydrophilic and nonpolar hydrophobic

		components
5	.... is used in burns	Silver sulfadiazine

- **Sterilizing Gases**
- Hexachlorophene Many heat-sensitive items such as disposable plastic Petri dishes and syringes, heart-lung machine components, sutures, and catheters are sterilized with ethylene oxide gas (figure 8.10) .
- Ethylene oxide (EtO) is both microbicidal and sporicidal. It is a very strong alkylating agent that kills by reacting with functional groups of DNA CH3-CH2-0H.
- Ethanol Isopropanol Halogenated compound Formaldehyde Cetylpyridinium Glutaraldehyde Benzalkonium Gases and proteins to block replication and enzymatic activity.
- It is a particularly effective sterilizing agent because it rapidly penetrates packing materials, even plastic wraps.
- Sterilization is carried out in an ethylene oxide sterilizer, which resembles an autoclave in appearance. It controls the EtO concentration,
- temperature, and humidity, Because pure EtO is explosive, it is usually supplied in a 10 to 20% concentration mixed with either C02 or dichlorodifluoromethane. The EtO concentration, humidity, and temperature influence the rate of sterilization. A clean object can be
- sterilized if treated for 5 to 8 hours at 38°C or 3 to 4 hours at 54°C when the relative humidity is maintained at 40 to 50% and the EtO concentration at 700 mg/L. Because it is so toxic to humans, extensive aeration of the sterilized materials is necessary to remove residual EtO.
- Betapropiolactone (BPL) is occasionally employed as a sterilizing gas. In the liquid form, it has been used to sterilize vaccines and blood products. BPL decomposes to an inactive form after several hours and is therefore not as difficult to eliminate as EtO. It also destroys micro-
- Ethylene oxide Hydrogen peroxide organisms more readily than ethylene oxide but does not penetrate materials well and may be Betapropiolactone carcinogenic. For these reasons, BPL has not Disinfectants and Antiseptics. The structures of some frequently used been used as extensively as EtO.
- Vaporized hydrogen peroxide (VHP) can also be used to decontaminate biological safety cabinets, operating rooms, and other large facilities.
- VHP is produced from a solution of disinfectants and antiseptics.

- Aldehydes

- Amongst the **aldehydes** (general formula R – CHO), several of the low **molecular** weight **aldehydes** are **antimicrobial**.
  Two **aldehydes**, **formaldehyde** and glutaraldehyde, are the most effective and are most commonly used to kill spores hence are sporicidal.

- **PHENOL COEFFICIENT METHOD FOR EVALUTION OF CHEMICAL ANTIMICROBIAL AGENTS**

- initial screening to see if they are effective and at what concentrations. This may be followed by more realistic in-use testing.
- The best-known disinfectant screening test is the phenol coefficient test in which the potency of a disinfectant is compared with that of phenol. A series of dilutions of phenol and the disinfectant being tested are prepared. Standard amounts of
- Salmonella enterica serovar Typhi and Staphylococcus aureus are added to each dilution; the dilutions are then placed in a 20 or 37°C water bath. At 5-minute intervals, samples are withdrawn from each dilution and used to inoculate a growth medium, which is incubated and examined for growth.
- Growth in the medium indicates that the dilution at that particular
- time of sampling did not kill the bacteria.
- The highest dilution (i.e., the lowest concentration) that kills the bacteria after a 10-minute exposure but not after 5 minutes is used to calculate
- the phenol coefficient.
- The higher the phenol coefficient value, the more effective the disinfectant under these test conditions.
- A value greater than 1 means that the disinfectant is more effective than phenol.
- The phenol coefficient test is a useful initial screening procedure, but the phenol coefficient can be misleading if taken as a direct indication of disinfectant potency during normal use.
- This is because the phenol coefficient is determined under carefully controlled conditions with pure bacterial cultures, whereas disinfectants are normally used on complex populations in the presence of organic matter and with significant variations in environmental factors such as pH, temperature, and presence of salts.
- To more realistically estimate disinfectant effectiveness, other tests are often used.

- The rates at which selected bacteria are destroyed with various chemical agents may be experimentally determined and compared. A use dilution test can also be carried out.
- Stainless steel carriers are contaminated with one of three specific bacterial species under carefully controlled conditions. The carriers are dried briefly, immersed in the test disinfectants for 10 minutes, transferred to culture media, and incubated for 2 days. The disinfectant concentration that kills the bacteria on at least 59 out of 60 carriers (a
- 95% level of confidence) is determined.
- Disinfectants also can be tested under conditions designed to simulate normal in-use situations. In-use testing techniques allow a more accurate determination of the proper disinfectant concentration for a particular situation.

➢ **Sr.no.**	**Question**	**Answer**
1	Full form of EtO	Ethylene oxide
2	Give full form of VHP	Vaporized hydrogen peroxide
3	EtO is reacting with functional group of.........	DNA CH3-CH2-0H.
4	.... is occasionally employed as a sterilizing gas	BPL
5	.... value indicate that disinfectant is more effective then phenol	Value grater then 1