The control of microorganisms

B.Sc.(Hons) Part 1

The term control as used refers to the reduction in numbers and /activity of the total microbial flora.

The principal reasons for controlling microorganisms are:

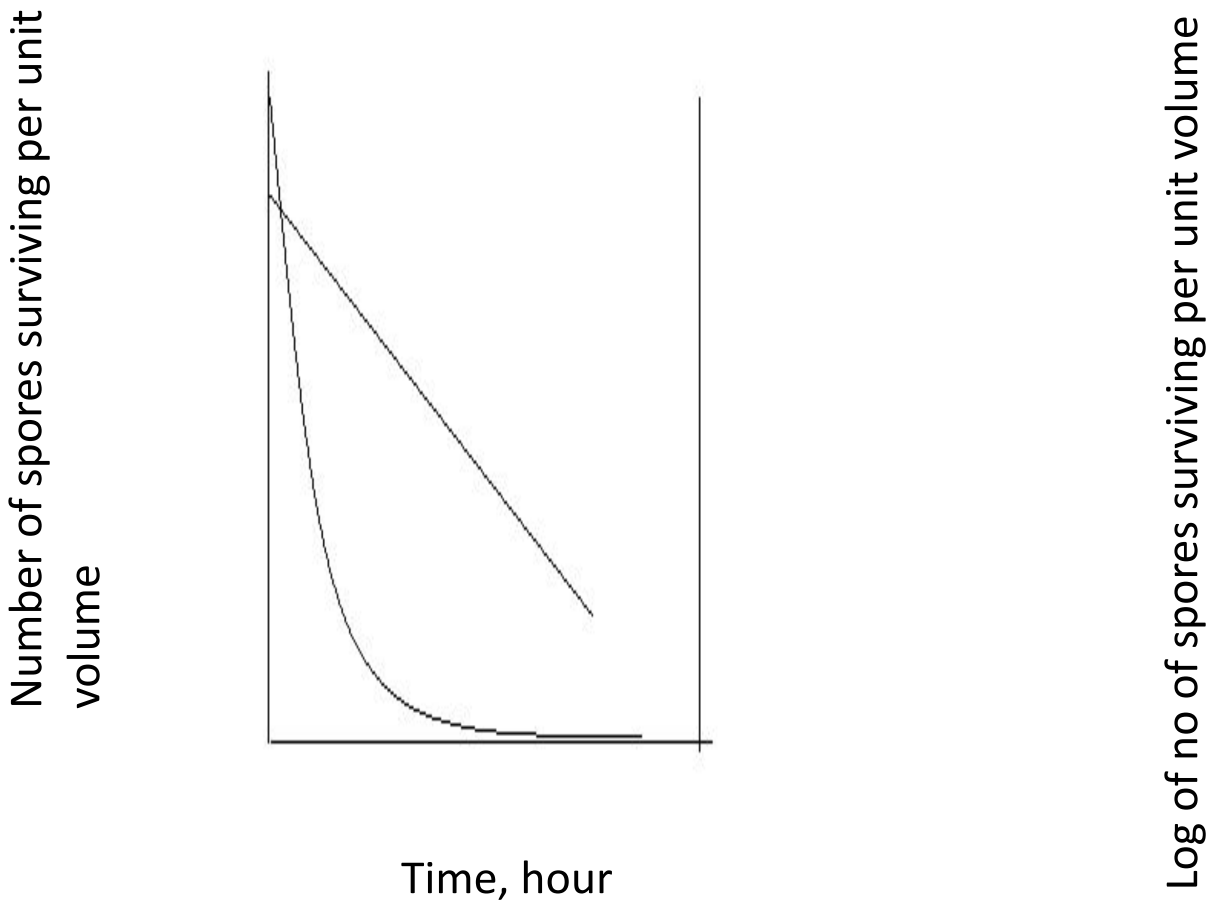
- ➢ To prevent transmission of disease and infection
- ➢ To prevent contamination by or growth of undesirable microorganisms
- ➢ To prevent deterioration and spoilage of materials by microorganisms

- ✓ Physical agent
- ✓ Physical processes
- ✓ Chemical agents

The term death as used in microbiology, is defined as the irreversible loss of the ability to reproduce. Viable microorganisms are capable of multiplying ; dead microorganisms donor multiply or grow. A complicating factor in this definition is that the response of the organism may not be the same in all media. e.g. *E.coli* exposed to heat may yield greater number of survivors if plated in trypticase soy broth rather than in medium containing bile salt such as deoxycholate agar.

The rate of death of bacteria

Time	Survivors	Deaths/unit time	Total Deaths
0	1,000,000	0	0
1	100,000	900,000	900,000
2	10,000	90,000	990,000
3	1,000	9,000	999,000
4	100	900	999,900
5	10	90	999,990
6	1	9	999,999



Number of spores surviving per unit volume
Time, hour
Log of no of spores surviving per unit volume

Conditions influencing antimicrobial action

It is not possible to prescribe one agent that will be effective for the control of microorganisms for all materials and all circumstances.

- **Environment**-The effectiveness of heat is much greater in acid than in alkaline material.
- **Kinds of microorganisms-** Species of microorganisms differ in their susceptibility to physical and chemical agents. In sporeforming species , the growing vegetative cells are much more susceptible than the spore forms ; bacterial spores are extremely resistant.
- **Physiological state of cells-** Young , actively metabolizing cells are apt to be more easily destroyed than old , dormant cells in the case of an agent that cause s damage through the interference with metabolism; nongrowing cells are not affected.

Mode of action of antimicrobial agents

- Damage to the cell wall or inhibition of cell-wall synthesis
- Alteration of the permeability of the cytoplasmic membrane
- Alteration of the physical or chemical state of proteins and nucleic acids
- Inhibition of enzyme action
- Inhibition of protein or nucleic acid synthesis

Physical agents

- High temperatures
- Low temperatures
- Desiccation
- Osmotic pressure
- Radiation
- Surface tension and interfacial tension
- Filtration

High temperatures

- Microorganisms can grow over a wide range of temperatures. Every type has an optimum, minimum and maximum growth temperature. Temperatures above the maximum generally kill , while below the minimum usually produce stasis (inhibition of metabolism) and may even be considered preservative.
- The amount of water present in the environment at any temperature has significant effect upon microorganisms in terms of their survival. High temperature along with high moisture is one of the most effective methods of killing microorganisms. Moist heat kills microorganisms by coagulating their proteins and is much more rapid and effective than dry heat, which destroys microorganisms by oxidizing their chemical constituents.

e.g. Spores of *Clostridium* are killed in 4- 20 mins @120 ^{0} C in moist heat, whereas in dry heat @ same temperature 2 hour s required.

- Vegetative cells are much more sensitive to heat than are spores; the higher level of "water activity" in the vegetative cells accounts for this. Cells of most bacteria are killed in 5-10min @ 60-70^0 C (moist heat). Vegetative cells of yeast and other fungi are usually killed 5-10 min by moist heat @ 50-60^0 C
- The susceptibility of viruses to heat is generally to that of mesophilic vegetative bacterial cells.
- Thermal death time refers to the shortest period of time to kill a suspension of bacteria (or spores) at a prescribed temperature and under specific conditions.
- Decimal reduction time is the time in minutes to reduce the population by 90%, or it is the time in minutes for the thermal-death-time curve to pass through one log cycle.

Practical procedures: moist heat & dry heat

1. <u>Moist heat</u>

- Steam under pressure - heat in the form of saturated steam under pressure is the most practical and dependable agent for sterilization. Steam under pressure provides temperatures above those obtainable by boiling. E.g. autoclave is used here.
- Fractional sterilization- This method involves the material at 100^0C on three successive days with incubation periods in between. Resistant spores germinate during the incubation periods; on subsequent exposure heat, the cells will be destroyed. If spores are present and do not germinate, the material will not be sterilized. E.g. steam arnold or an autoclave with free flowing steam can be used here.
- Boiling water – All vegetative cells will be destroyed, but not all bacterial spores. It can surely cause disinfection, rather than sterilization.

- Pasteurization – Can be used for milk, cream and certain alcoholic beverages (like beer and wine). Kills microorganisms of certain types but does not destroy all organisms. Pasteurized milk is not sterile milk. 2 methods of pasteurization- LTH or low-temperature-holding method and HTST or high-temperature-short-time method.

LTH -62.8 ^{0}C for 30 minutes; HTST – 71.7 ^{0}C for 15 seconds. Precautions must be taken to prevent recontamination after pasteurization. The finished product should be stored at a low temperature to retard growth of microorganisms which survived pasteurization.

Radiation

Most significant radiation- ionizing radiation, e.g. light and x-ray

Electromagnetic radiation – dual properties of a continuous wave function and a discontinuous particle phenomenon.

Ionizing radiation- knock electrons away from molecules and ionize them.

Cell→ radiation→ free hydrogen radicals, hydroxyl radicals and some peroxides → different kinds of intracellular damage (nonspecific)

UV- less energetic, specific- excites electrons and raise them to a higher energy level→ diff chemical species → variety of chemical reaction , not possible for unexcited molecules

Cold sterilization- sterilization by ionizing radiation –less heat generated—useful for heat sensitive materials, used mainly in food and pharmaceutical industries.

UV→ 150- 3900A° , 2650 A° highest bactericidal efficiency, very little penetration capacity, so only surface sterilization. Cause thymine dimers

Germicidal lamps – used in hospital operating rooms, in aseptic filling rooms, in the pharmaceutical industry, where sterile products are dispensed vials or ampules, in food and dairy industries for treatment of contaminated surfaces.

X-ray & Gamma ray

X ray- lethal to microorganisms & higher organisms, more energy an penetration capacity than UV. They are very impractical to use because- 1. very expensive, 2. utilization not easy as it radiates in all directions. Widely used for experiments to produce microbial mutants

Gamma- high energy radiation, shorter wavelength than x ray, lethal to all organisms, great penetration

Use in commercial sterilization of materials of considerable thickness and volume e.g. packaged food and medical devices

Cathod rays- microbicidal effects, limited power of penetration, but can be sterilized with brief exposure, materials can be sterilized after packaging

Lethal doses of different radiations, mrd

Types of organisms	Cathod rays from van de Graaff Accelerators	From Capacitron Pulsed Beam	Gamma rays from ^{60}Co	X rays from 3-Mev Source
Vegetative:				
Nonpathogenic	0.1-0.25			
Pathogenic	0.45-0.55	0.1-0.25	0.15-0.25	0.03-0.5
Bacterial spores	0.5-2.1	0.2-0.4	~ 1.5	0.5-2.0
Molds	0.25-1.15	0.35-0.4	0.2-0.3	0.25-1.0
Yeasts	0.5-1.0		0.3	0.25-1.5

Surface tension & interfacial tension

The interface or boundary, between a liquid and a gas is the characterized by unbalanced forces of attraction between the molecules in the surface of the liquid and in the interior. A molecule at the surface of the liquid-air interface is pulled strongly toward the interior of the liquid beneath it, whereas molecules in the interior of the liquid are attracted uniformly in all directions. This behavior of molecular forces at the liquid-air interface imparts a distinctive characteristics to the surface of a liquid, known as surface tension. Surface forces also exist between two immiscible liquids and at the interface between a solid and a liquid. Here they are referred to as interfacial tension. Changes in surface tension may alter the permeability characteristics of the cytoplasmic membrane, causing leakage of cellular substances, which results in damage to the cell.

Filtration

Can remove bacteria from liquid and gases

Materials: asbestos pad in the Seitz filter, diatomaceous earth in the Berkefeld filter, porcelain in the Chamberland-Pasteur filter and sintered glass disks in other filters.

Mean pore diameter: one to several micrometers; most filters are available in several grades based on the average size of the pore.

Porosity, electric charges of the filter, electric charges carried by the organism, nature of the fluid to be filtered- influence efficiency of filtration

Membrane or molecular filter – pores are uniform, made up of biologically inert cellulose ester. They are prepared as circular membranes of about 150µm thickness and contain millions of microscopic pores of uniform diameter. Porosity ranges from 0.01- 10 µm. Used extensively in labs & in industries to sterilize fluid materials.

Fluid is forced through membrane by –ve pressure to te filter flask by use of a vacuum or water pump or by imposing +ve pressure above the fluid in the filter chamber, thus forcing it through.

HEPA(high efficiency particulate air) filter-used in Laminar Air Flow Hood

Control by Chemical Agents

There is not a single chemical agent which is best for the control of microorganisms for any or all purposes.

Characteristics of an ideal antimicrobial chemical agent:

1. **Antimicrobial activity-** @ a low concentration should have a broad spectrum of antimicrobial activity
2. **Solubility-** should be soluble in water or other solvents to the extent necessary for effective use
3. **Stability-** changes @ standing should be minimal and no change of antimicrobial activity
4. **Nontoxicity to humans and other animals-** lethal to microorganisms and non injurious to humans & other animals
5. **Homogeneity-** uniform in composition so that active ingredients are present in each application
6. **Noncombination with extraneous organic material-** affinity of chemical agent for protein or other organic material may lead to nonavaibility of the antimicrobial agent for action against microorganisms
7. **Toxicity to microorganisms at room or body temperature-** temperature of action and temperature of target microorganism should not be very different

8. Capacity to penetrate-

9. Noncorroding and nonstaining-

10. Deodorizing capacities- either odourless or with pleasant smell

11. Detergent capacities-

12. Availability- available in large quantities @ a reasonable price

Some terms

Sterilization- The process of destroying all sorts of microbial life. A sterile object, in the microbiological sense, is free of living microorganisms. This term and related terms should not be used in relative sense, i.e. an object/ substance is sterile or nonsterile, it can never be semi sterile or almost sterile.

Disinfectant- An agent, usually a chemical, that kills the growing forms but not necessarily, the resistant spore forms of disease-producing microorganisms. The term is commonly applied to substances used on inanimate objects. Disinfection is the process of destroying infectious agents.

Antiseptic- A substance that opposes sepsis i.e. prevents the growth or action of microorganisms either by destroying microorganisms or by inhibiting their growth and metabolism. Usually associated with substances applied to the body.

Sanitizer- An agent that reduces the microbial population to safe levels as judged by public health requirements. Usually it is a chemical agent that kills 99.9% of the growing bacteria. Commonly applied to inanimate objects, daily care of equipment & utensils in dairies and food plants for glasses, dishes and utensils in restaurants. disinfection produce→sanitization; in the strict sense sanitization implies a sanitary condition which disinfection does not necessarily imply.

Germicide (**Microbicide**)- An agent that kills the growing forms but not necessarily the resistant spore form of germs; in practice a germicide is almost the same thing as a disinfectant, but germicides are commonly used for all kinds of germs (microbes) for any application.

Bactericide- An agent that kills bacteria(adjective, bactericidal). Similarly, the terms fungicide, virucide and sporicide refers to agents that kill fungi, viruses, and spores, respectively.

Bacteriostasis- A condition in which the growth of bacteria is prevented (adjective, bacteriostatic). Similarly fungistatic describes an agent that stops the growth of fungi. Agents that have in common the ability to inhibit growth of microorganisms are collectively designated microbistatic agents.

Antimicrobial agent – One that interferes with the growth and metabolism of microbes. In common usage the term denotes inhibition of growth , and with reference to specific groups of organisms such as antibacterial or antifungal are frequently employed. Some antimicrobial agents are used to treat infections, and they are called chemotherapeutic agents.

SELECTION OF A CHEMICAL AGENT FOR PRACTICAL APPLICATIONS

1. **Nature of the material to be treated-** e.g. chemical agent needed to disinfect utensils is not compatible for human skin.
2. **Types of microorganisms**- Chemical agents are not equally effective against bacteria, fungi, viruses and other microorganisms. Spores are more resistant than vegetative cells. Differences exist between Gram- +ve and Gram –ve bacteria; *Escherichia coli* is much more resistant to cationic disinfectants than *Staphylococcus aureus*. Differences in action also exist between strains of the same species. Therefore, the agent selected must be known to be effective against the type of organism to be destroyed.
3. **Environmental conditions-** Factors like temperature, pH, time, concentration and presence of extraneous organic material, may all have a bearing on the rate and efficiency of antimicrobial action. The successful use of an antimicrobial agent requires understanding of the influence of these conditions on the particular agent, so it can be employed under the most favorable circumstances.

Major groups chemical agents

- Phenolic and phenolic compounds
- Alcohols
- Halogens
- Heavy metals and their compounds
- Dyes
- Detergents
- Quaternary ammonium compounds
- Aldehydes
- Gaseous agents

Phenol & phenolic compounds

Phenol has been used in 1880s by Joseph Lister, a surgeon, to reduce infection of surgical incisions and surgical wounds. He was aware of Pasteur's studies which incriminated germs as the cause of infection. Accordingly he used a solution of phenol (carbolic acid) to surgical incisions & wounds. The reduction in infections was striking. Later he developed the practice of spraying phenol into the operating room area to control infection.

Phenol has the additional distinction of being the standard against other disinfectants of a similar structure & are compared to determine their antimicrobial activity. The procedure used is called the phenol-coefficient technique.

Phenol & phenolic compounds are very effective disinfectants. A 5% aqueous solution of phenol rapidly kills the vegetative cells of microorganisms; spores are much more resistant. It has been seen that antimicrobial activity increases by the addition of chemical substitutions in the phenol structure.

e.g. Hexylresorcinol – a derivative of phenol, marketed in a solution of glycerin and water. It is strong surface-tension reductant, which may account partly for its high bactericidal activity.

Practical applications & mode of action

Phenolic substances either bacteriostatic or bactericidal, depending upon the concentration used. Spores are more resistant than vegetative cells. Some phenolics are highly fungicidal. The antimicrobial activity reduces at alkaline pH, by organic material, low temperature and the presence of soap. 2-5% aqueous solution is able to disinfect sputum, urine, feces & contaminated utensils and instruments.

Solutions of pure phenol is of limited use. One of the widely used derivative is o-phenylphenol- its compound combined with detergents result in product of good disinfectant as well as detergent properties.

Mode of action Depending upon the concentration used, resultant effect can be disruption of cells, precipitation of cell protein, inactivation of enzymes, leakage of amino acids from the cells. Although the specific mode of action is not clear, there is a consensus that the lethal effect is associated with the physical damage of the membrane on the cell surface, which initiates further detoriation.

Alcohol

Ethyl alcohol CH_3CH_2OH (50%-90%) effective against vegetative or nonsporeforming cells. For practical purposes 70% is used. It cannot produce sterile condition, concentration effect against vegetative cells are practically inert against bacterial spores.

Methyl alcohol is less bactericidal than ethyl alcohol; it is highly poisonous. Fumes can cause permanent damage to the eyes; generally not used for bacterial destruction. The higher alcohols – propyl, butyl, amyl and others are more germicidal than ethyl alcohol. There is a progressive increase of germicidal power with increase in mol. wt. Alcohols of molecular weight higher than propyl alcohol are not miscible in water in all proportions so they are not used as disinfectant. Propyl alc and isopropyl alc are bactericidal for vegetative cells at 40-80% concentrations.

Phenol coefficient of n-Octyl is biggest whereas Methyl alcohol is the smallest among alcohols.

Practical applications & mode of action of alcohol

Alc is effective against reducing the microbial of skin & disinfection of oral thermometer. Alc conc >60% is effective against viruses, however it is influenced by extraneous protein material in the mixture. The protein reacts with alc & protects virus.

Alc are protein denaturants- accounts largely for its antimicrobial action. It is a solvent of lipid thus damage lipid in the cell membrane. They are dehydrating agent- alc is relatively ineffective in dry cells. Very thy high conc of alc dry the cells in such a way that alc cannot penetrate the cell, resulting in bacteriostatic condition. Mechanical removal of microorganisms in cleansing or detergent action.

Halogens

Iodine- most oldest & effective germicidal. Used for more than a century, by US Pharmacopoeia in 1830 . bluish-black crystalline element with metallic luster, slightly soluble in water, but readily in alc and aqueous soln of NaI & K. traditional germicidal- **tincture of iodine.** Several preperations such as 2% Iodine+ 2% NaI diluted in alc; 7% iodine + 5% KI in 83% alc; 5% iodine + 10% KI in aqueous soln are available. Used as substance called **iodophors.** It is a mixture of iodine & surface active agents acting as carrier & solubilizers of iodine, e.g. polyvinylpyrrolidone(PVP). Complex is expressed as PVP-1. Slowly releasing Iodine acts as germicidal and non staining & low irritant properties.

Practical applications- highly effective <u>bactericidal</u> agent, effective against all kinds of bacteria. It is also <u>sporicidal</u>- rate of action depends on amount of organic and extent of hydration. It is highly <u>fungicidal</u> and to some extent <u>virucidal</u>. Iodine soln is chiefly used for disinfection of skin –rank among the best disinfectants. It is also used for disinfection of water, air (iodine vapour) and sanitization of food utensils.

Mode of action

Mechanism is not specifically understood. It is an oxidizing agent- accounts for its antimicrobial action. They irreversibly & inactivate essential metabolic compounds like proteins with sulfhydryl group. It is suggested that the action may involve the halogenation of tyrosine units of enzymes & other cellular proteins requiring tyrosine for activity.

CHLORINE

In the form of gas or in certain chemical combinations, represents one of the most widely used disinfectants. The compressed gas in liquid form is almost universally employed for the purification of municipal water supplies. Chlorine gas difficult handle unless special equipment is available to dispense it. Hence its usefulness in the gaseous state is limited to large scale operations such as water purification plants, where it is feasible for installing suitable equipments for safe handling.

There are available many compounds of chlorine which can be handled more conveniently than free chlorine and which, under propoer conditions of use, are equally effective as disinfectants. One class of compounds in this category is the hypochlorites. Calcium hypochlorite, $Ca(OCl_2)$ (also known as chlorinated lime), and sodium hypochlorite, $NaOCl$, are popular compounds. The chloramines represents another category of chlorine compounds used as disinfectants, sanitizing agents, or antiseptics. Chemically they are characterized by the fact that one or more of the hydrogen atoms in in an amino group of a compound are replaced by chlorine. The simplest is monochloramine, NH_2Cl. The advantage of chloramine over hypochlorites is its stability, in terms of prolonged release of chlorine.

Semmelweis (1846-48) → used hypochlorite to reduce risk of childbed fever. Medical students were required to wash their hands and soak them in a hypochlorite solution before examining patients.

Chlorine compounds→ water treatment, food industry, domestic use, medicine. Disinfect open wounds, to treat athletes foot 7 other infections and as general disinfectants.

Products containing calcium hypochlorite → sanitizing dairy equipments, eating utensils in restaurants.

1% solution of sodium hypochlorite→ personal hygiene, household disinfectants

5-12% solution of sodium hypochlorite→ household bleaches and disinfectants; sanitizing agents in diary and food processing establishments.

The amount of hypochlorite added should provide a residual concentration of approximately 1mg/litre of free chlorine.

Mode of action

(free) $Cl_2 + H_2O \rightarrow HCl + HClO$ (hypochlorous acid)→ antimicrobial

$HClO \rightarrow HCl + O$ (formed from chlorine, hypochlorites, chloramines)

This O is nascent oxygen→ is a strong oxidizing agent→ acts on cellular constituents & kills cells. Also chlorine combine directly with protein of cell membrane and enzymes and kills cells.

Heavy metals & their compounds

Control by Chemical Agents

Heavy Metal	Examples of Compounds	Applications
Mercury	Inorganic compounds: Mercuric chloride (bichloride of mercury) Mercurous chloride Mercuric oxide Ammoniated mercury	Bactericidal in dilutions of 1:1,000; limited use because of corrosive action, high toxicity to animals, and reduction of effectiveness in presence of organic material; insoluble compounds, used in ointments as antiseptics
	Organic compounds: Mercurochrome Metaphen Merthiolate Mercresin	Less irritating and less toxic than the inorganic mercury compounds; employed as antiseptics on cutaneous and mucosal surfaces; may be bactericidal or bacteriostatic
Silver	Colloidal silver compounds: Silver nitrate Silver lactate Silver picrate	Consist of protein in combination with metallic silver or silver oxide (colloidal solution); bacteriostatic or bactericidal effect is a function of the free silver ions released from the combination; used as antiseptics, silver nitrate is the most widely used of these compounds, all of which are germicidal and employed as antiseptics in specific conditions; silver nitrate is bactericidal for most organisms at a dilution of 1:1,000; many states require that the eyes of newborns be treated with a few drops of 1% silver nitrate solution to prevent ophthalmia neonatorum, a gonococcal infection of eyes
Copper	Copper sulfate	Much more effective against algae and molds than bacteria; 2 ppm in water sufficient to prevent algal growth; used in swimming pools and open water reservoirs; used in the form of Bordeaux mixture as a fungicide for prevention of certain plant diseases

Most of the heavy metals, either alone or in certain compounds, exert a detrimental effect upon microrganisms. The most effcetive are mercury, silver and copper.

Mode of action Heavy metals and their compounds act antimicrobially by combining with cellular proteins and inactivating them. E.g. Mercuric chloride→inhibits enzymes containing sulfhydryl group.

$$\text{Enzyme}\begin{cases}\text{SH}\\ \text{SH}\end{cases} + HgCl_2 \rightarrow \text{enzyme}\begin{cases}\text{S}\\ \text{S}\end{cases}\!\!\!>\text{Hg} + 2HCl$$

Active enzyme Mercuric chloride Inactive enzyme

High concentrations of salts of heavy metals like mercury, copper and silver coagulate cytoplasmic proteins, resulting in damage or death of the cells. Salts of heavy metals are also precipitants, and in high concentrations such salts could cause death of the cells.

Dyes

Two types of dyes: timethylmethane and acridine dyes

Timethylmethane include malachite green, brilliant green and crystal violet. As a rule, Gram positive organisms are more susceptible to lower concentrations of these compounds than are Gram-negative ones. Crystal violet will inhibit Gram-positive cocci at at dilution of 1: 200,000 to 1: 300,000; 10 times this concentration is required to inhibit *E.coli*. *Staphylococcus aureus* is inhibited by malachite green at a conc. Of 1: 1,000,000; a conc. of about 1: 30,000 is required to inhibit *E.coli*.

Practical application: certain media at low concentration (1: 100,000) of dye crystal violet, brilliant green or malachite green can inhibit Gram positive bacteria, used extensively in public health microbiology, where detection of *E.coli* is important. 3 species of *Brucella* can be distinguished by their patterns of resistance to several dyes. Crystal violet can also be used as a fungicide. A conc. of 1;10,000 is lethal for *Monilla* and *Torula*, and a conc. of 1:1,000,000 is inhibitory.

Mode of action is uncertain but speculation is that it interferes withcellular oxidation preocess.

Acridine dyes

Example – acriflavine & tryptoflavine

Exhibit selective inhibition against bacteria, particularly staphylococci and gonococci. Gonococci are inhibited by tryptoflavine in dilutions of 1:10,000,000 to 1:50,000,000. They have very little if any antifungal activity. They were mostly used before the antibiotics era. They are used to some extent for the treatment of burns and wounds and for ophthalmic application and bladder irrigation.

Synthetic detergents

Surface- tension depressants or wetting agents, employed primarily for cleansing surfaces are called **detergents**. e.g. soap., which is a poor detergent. For this reason many new more efficient cleaning agents have been developed, called surfactants or synthetic detergents, superior to soap. They donot form precipitates in alkaline or acid water, nor they produce deposits with minerals found in hard water. Uses are laundry, dishwashing powders, shampoos and other washing preparations. Some are highly bactericidal. They are classified as anionic, cationic and nonionic.

1. Those which ionize with the detergent property resident in the anion are anionic detergent. $[C_9H_{19}COO]^- Na^+$ →a soap, $[C_{12}H_{25}OSO_3]Na^+$ → sodium lauryl sulfate
2. Those which ionize with the detergent property resident in the cation are cationic detergent. Cetylpyridinium chloride (Ceepryn)
3. Those which do not ionize are called nonionic detergent.

Ordinary soaps mechanical removal of microorganisms.Soaps reduce surface tension and thereby increase the wetting power of the water in which they are dissolved. Soapy water emulsify and disperse oils and dirt. The microorganisms get enmeshed in the soap lather and are removed by the rinse water. Various chemicals enhance the germicidal activity of soap. Cationic detergents are regarded as more germicidal than anionic compounds.

Quaternary Ammonium Compounds

Most compounds of the germicidal cationic-detergent class are quaternary ammonium salts. $NH_4Cl \rightarrow$ H is replaced by carbon groups (R), linked with the N atom. R may be different alkyl groups.

Antibacterial activity is exceptionally high against Gram + ve bacteria, and quite high against Gram –ve ones. They have more bacteriostatic than bactericidal. Examples are Cetrimide, Ceepryn, Zephirol etc. They have also been shown to be fungicidal and lethal to some protozoans. Viruses are more resistant than bacteria and fungi.

Practical applications The combined properties of germicidal activity and detergent action plus such other features as low toxicity, high solubility in water, stability in solution, and noncorrosiveness, have resulted in many applications as disinfectants and sanitizing agents. They are used as skin disinfectant, as preservative in ophthalmic solution and in cosmetic preperation. Widely used as for control of microorganisms in floors, walls, and other surfaces in hospitals, nursing homes and other public places. They are used in sanitizing utensils in restaurants and food processing plants. Other applications are found in the dairy, egg and fishing industries to control microbial growth on surfaces of equipments and the environment in general.

Mode of action

A variety of damaging effects can be observed like denaturation of proteins, interference with glycolysis and membrane damage. The cytoplasmic membrane is damaged by these compounds.

Aldehydes

Among the class of chemicals with the general formula RCHO (aldehydes), several of the low molecular weight compounds are antimicrobial. Two of the most effective are formaldehyde and glutaraldehyde. Both are highly antimicrobial and can kill spores.

Formaldehyde simplest form of aldehyde, stable only in high concentrations and at elevated temperatures. At room temperature it polymerases to a solid substance. The important polymer is paraformaldehyde, a colourless substance which rapidly yields formaldehyde upon heating. Formaldehyde is sold as aqueous solution as formalin (37-40% formaldehyde). The tissues and eyes get irritated by the noxious fumes of formaldegyde.

Practical applications

Formaldehyde in solution used for sterilizing certain instruments. In gaseous form can be used for disinfection and sterilisation of areas. Principal sources of formaldehyde are formalin and paraformaldehyde for gaseous disinfection. Vegetative cells are killed more quickly than spores. Humidity and temperature have a pronounced effect on the antimicrobial action of formaldehyde; temperature of the enclosure should be around 22^0 C and relative humidity between 60-80%. This vapour cannot penetrate covered surfaces.

Mode of action it is an extremely reactive molecule. It combines readily with vital organic nitrogenous compounds like proteins and nucleic acid, which likely is the reason for its antimicrobial action.

GLUTARALDEHYDE

Glutaraldehyde is a saturated dialdehyde with the formula

$O=C-CH_2-CH_2-CH_2-C=O$

$\quad |\qquad\qquad\qquad\qquad |$

$\quad H\qquad\qquad\qquad\qquad H$

A 2% solution of this chemical agent exhibits a wide spectrum of antimicrobial activity. It is effective against vegetative bacteria, fungi, bacterial and fungal spores and viruses. It has use in medical fields in sterilizing urological instruments, lensed instruments, respiratory therapy equipment and other special equipments.

Gaseous agent

It is effective for agents that are heat sensitive, like plastic syringes, blood transfusion apparatus, etc. Ethylene oxide, beta-propiolactone, and formaldehyde.

ETHYLENE OXIDE – H_2C-H_2C

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It is a liquid at temp below 10.8 ^{0}C (51.4 ^{0} F). At it , it vaporizes rapidly. Vapours of this compound in air in low concentration is also very inflammable like diethyl ether. This can be overcome by making mixture with carbon dioxide or Freon, without altering the antimicrobial action, available commercially. Ethylene oxide is a unique and powerful sterilizing agent used for heat or moisture sensitive material in hospitals, industry and laboratories. Bacterial spores show little resistance to destruction by this agent. An outstanding feature is its penetrating power. It will pass through large packages of materials, bundles of cloth and even certain plastics. It should be used with caution. The commercially available apparatus to use this agent is an autoclave filled with is gas under controlled conditions. The conc of ethylene oxide, temperature and humidity determines the time required for sterilizaton. Modern autoclaves control ethylene oxide conc , humidi y and temp.

Mode of action

The mode of action of ethylene oxide is believed to be alkylation reactions with organic compounds such as enzymes and other proteins. Alkylation consists in the replacement of an active hydrogen atom in an organic compound, with an alkyl group. In this the ring in the ethylene oxide molecule splits and attaches itself where the hydrogen was originally. This reaction would inactivate an enzyme with a sulfhydryl group:

H_2C----H_2C + enzyme-SH→enzyme-S-CH_2CH_2OH(inactive)

\ /

O

This compound is a colorless liquid at room temperature with a high boiling point (155^{0}C) and has the formula CH2--CH2

O------C O

β- Propiolactone

It is not flammable like ethylene oxide but is a vesicant and lachrymator and consequently must be handled with care. It lacks the penetrating power of ethylene oxide but is considerably more active against microorganisms; it is sporicidal, virucidal and fungicidal. Whereas the usual concentration of ethylene

Oxide for sterilization purpose is 400- 800 mg/litre, only 2-5 mg/ litre is required for β- Propiolactone. It is very active in destroying microoranisms on surfaces. However the fact that it has a low power of penetration coupled with its alleged carcinogenic properties has restricted its use as a practical sterilising agent.

AOAC phenol coefficient method or FDA method (**A**ssociation of **O**fficial **A**gricultural **C**hemists and **F**ood and **D**rug **A**dministration)

This procedure is suitable for testing disinfectants miscible with water and exerting their antimicrobial action in a manner similar to that of phenol. The test organism is either *Salmonella typhi* or *Staphylococcus aureus*.

(1) A series of dilutions of test disinfectant (5 ml/tube) + 0.5 ml of 24 hr broth culture of test organism.

(2) A series of dilutions of phenol (5 ml/tube) + 0.5 ml of 24 hr broth culture of test organism.

Both (1) & (2) were placed at 20^0C

↓

At intervals 5, 10, 15 minutes subcultures were made into sterile media.

↓

Incubated at suitable temperature and checked for growth.

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The greatest dilution of the disinfectant killing test organism in 10 min but not in 5 min is divided by the greatest dilution of phenol showing the same result =PHENOL COEFFICIENT

Chemical agent	Recommended use	Limitations
Phenol and phenolic compounds	General disinfectant	Microbial effectiveness limited; irritating & corrosive
Alcohols: ethyl & isopropyl	Skin and thermometer antiseptic	Antiseptic
Iodines	Disinfect skin	Irritating to mucous membrane
Chlorine	Water disinfection	Inactivated by organic material; pH dependent for effectiveness; objectionable taste & odor unless contolled
Silver nitrate	Treating burns	Possible irritation
Mercurials	Skin disinfection	Slow acting; toxic
Quaternaries	Skin disinfection	Not sporicidal

Chemical agent	Recommended use	Limitations
Formaldehyde	Sterilizing instruments; fumigation	Permeation poor; corrosive
Glutaraldehyde	Sterilizing instruments; fumigation	Stability limited
Ethylene oxide	Sterilizing heat sensitive materials, instruments and large equipment	Flammable; potentially explosive in pure form
β- Propiolactone	Sterilizing instruments and heat sensitive materials	Lacks penetating power