MEDICINAL CHEMISTRY

Chemotherapy: Antiseptics and Disinfectants

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Antiseptic, disinfectant, biocide, anti-infective, dyes, quaternary ammonium compounds, phenol, alcohol, formaldehyde, chlorhexidine, iodine, hydrogen peroxide

Introduction

Anti-infective agents or germicides or biocides may be broadly classified as Antiseptics and Disinfectants. They are used to kill or restrict the growth of micro-organisms when applied to a living tissue or inanimate objects. An antiseptic is a chemical compound that kills or inhibits the growth of micro-organisms when applied to a living tissue like skin etc., while a disinfectant is a chemical compound that prevents infection by the destruction of harmful micro-organisms when applied to inert objects like forcep, bedding, floor etc.

They should possess the following properties-

1. Chemical stability
2. Economical
3. Non-staining with acceptable colour and odour.
4. Bactericidal, not only static but capable of destroying spores as well.
5. Wider spectrum of action.

An antiseptic in addition should be-

1. Rapid in action and exert sustained lethal action.
2. Non-irritating to tissues when applied.
3. Non-allergic to the subject.
4. No systemic toxicity (Non-absorbable).
5. Active even in the presence of body fluids e.g.- blood, pus.

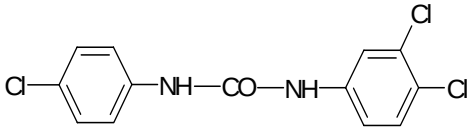
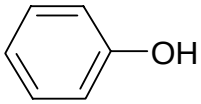
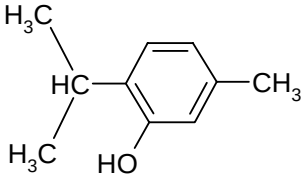
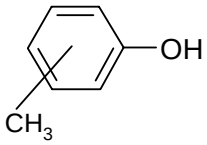
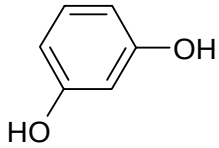
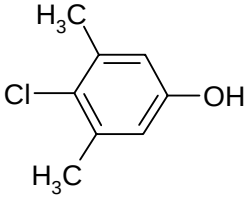
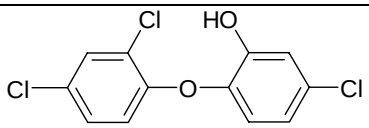
A disinfectant in addition should be-

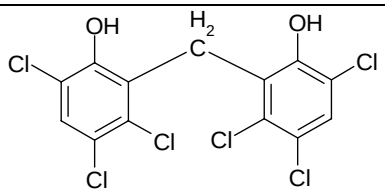
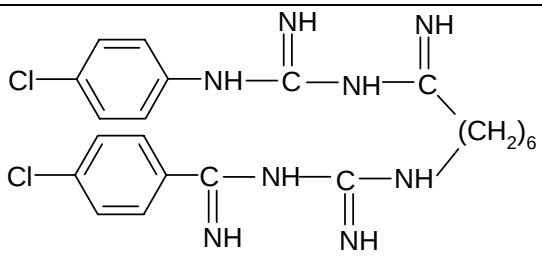
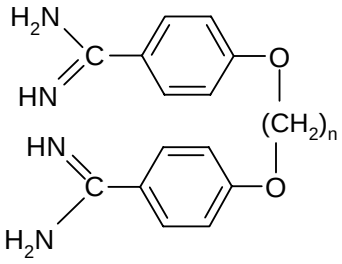
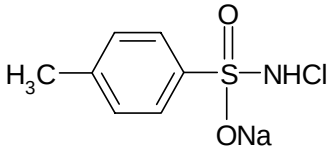
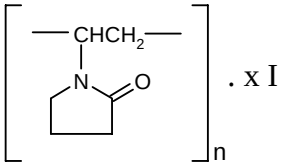
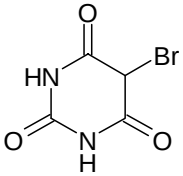
1. Non-corrosive
2. Good penetrating agent
3. Compatible with other organic compounds like soap.

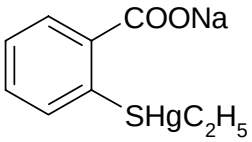
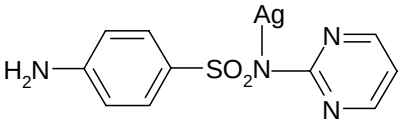
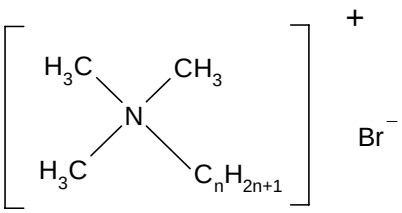
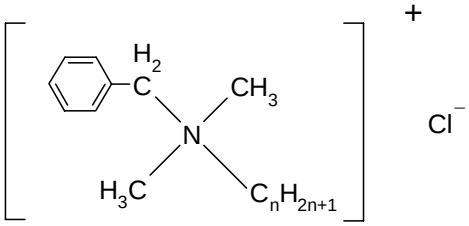
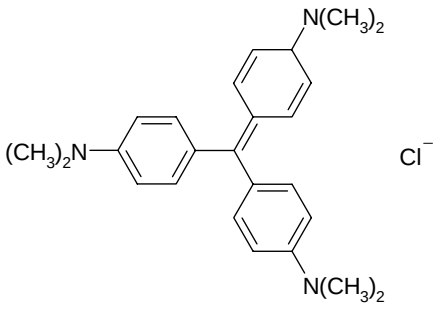
Antiseptics and disinfectants are used extensively in hospitals and other health care settings for a variety of topical and hard-surface applications. In particular, they are an essential part of infection control practices and aid in the prevention of nosocomial infections. Mounting concerns over the potential for microbial contamination and infection risks in the food and general consumer markets have also led to increased use of antiseptics and disinfectants by the general public. A wide variety of active chemical agents (or "biocides") are found in these products, many of which have been used for hundreds of years for antisepsis, disinfection, and preservation. Despite this, less is known about the mode of action of these active agents than about antibiotics. In general, biocides have a broader spectrum of activity than antibiotics, and, while antibiotics tend to have specific intracellular targets, biocides may have multiple targets. The widespread use of antiseptic and disinfectant products has prompted some speculation on the development of microbial resistance, in particular cross-resistance to antibiotics.

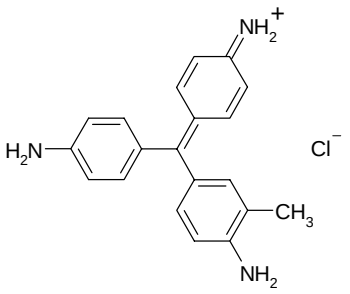
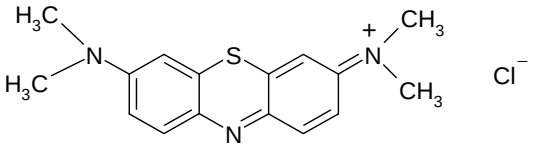
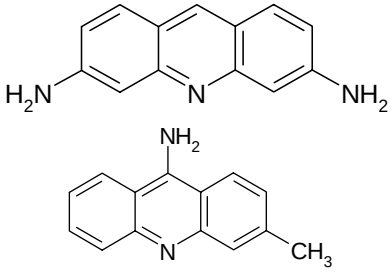
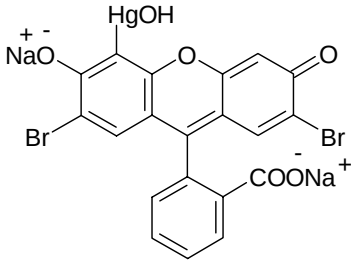
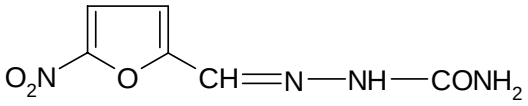
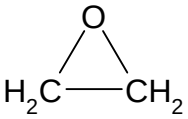
A summary of the various antiseptics and disinfectants with their chemical structure and clinical uses is shown in the **table-1**. It is important to note that many of these biocides may be used singly or in combination in a variety of products which vary considerably in activity against microorganisms. Antimicrobial activity can be influenced by many factors such as formulation effects, presence of an organic load, synergy, temperature, dilution, and test method.

Table-1: Antiseptics and disinfectants with their structure and uses

Class	Name	Structure	Uses/ Application
Alcohols	Ethanol Isopropanol	$CH_3—CH_2OH$ CH_3, CH_3, CHOH, CH_3	Antisepsis Disinfection Preservation
Aldehydes	Gluteraldehyde	$CHOCH_2CH_2CH_2CHO$	Disinfection Preservation Sterilization
	Formaldehyde	HCHO	
Anilides	Triclocarbon		Antisepsis
Phenols	Phenol		Antisepsis Disinfection Preservation
	Thymol		
	o, p & m-Cresol		
	Resorcinol		
	Chloroxylenol		
Bisphenols	Triclosan		Antisepsis Antiplaque Deoderants Preservation

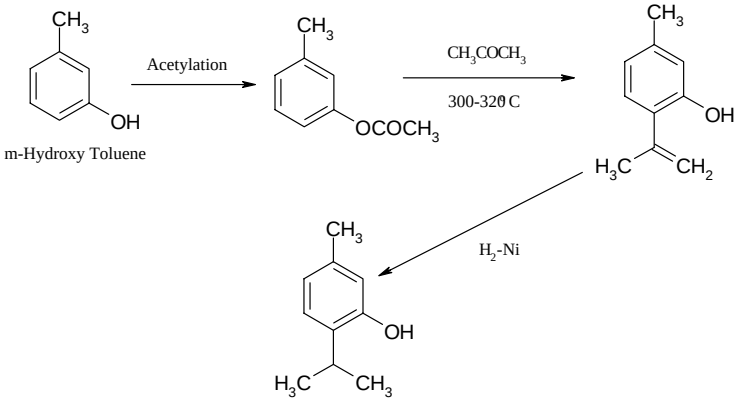
	Hexachlorophene		
Biguanides	Chlorhexidine		Antisepsis Antiplaque Disinfection Preservation
Amidines	Propamidine		Antisepsis Preservation
Halogen releasing agents	**Chlorine compounds** Hypochlorous acid Choramine	HOCl 	Antisepsis Disinfection Cleansing
	Iodine compounds Iodine Povidone iodine	I_2 	
	Bromine compounds Dibromin		
Heavy metals	**Mercury compounds** Mercuric	$HgCl_2$	Antisepsis Preservation Disinfection

	chloride Thiomersal		
	Silver compounds Silver nitrate Silver sulfadiazine	$AgNO_3$ 	
	Zinc compounds Zinc sulphate Zinc oxide	$ZnSO_4$ ZnO	
Per-oxygens	Hydrogen peroxide Ozone Peracetic acid	H_2O_2 O_3 CH_3COOOH	Disinfection Sterilization
Quaternary ammonium compounds	Cetrimide		Antisepsis Preservation Disinfection Cleansing
	Benzalkonium chloride		
Dyes	**Triphenyl-methane dyes** Gentian violet		Disinfection

	Basic Fuchsine		
	Thiazine dyes Methylene blue		
	Acridine dyes Acriflavin Salacrin		
	Xanthine dyes Mercurochrome		
Acids	Boric acid Acetic acid	H_3BO_3 CH_3COOH	Preservation
Furan derivatives	Nitrofuran		Antisepsis
Oxides	Ethylene oxide		Sterilization Disinfection

Synthesis of some important antiseptics and disinfectants

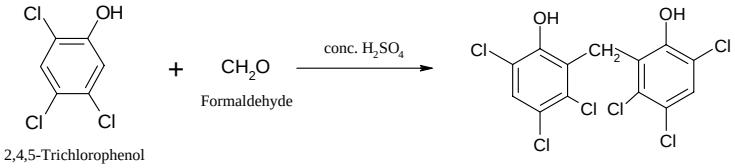
1. Synthesis of Thymol



m-Hydroxy Toluene

Thymol

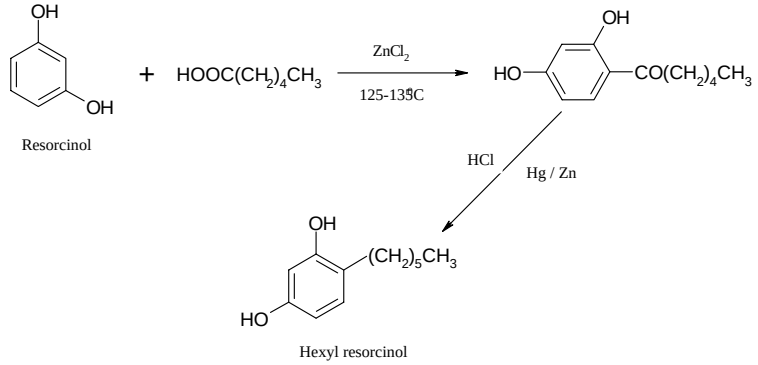
2. Synthesis of Hexachlorophene



2,4,5-Trichlorophenol

Hexachlorophene

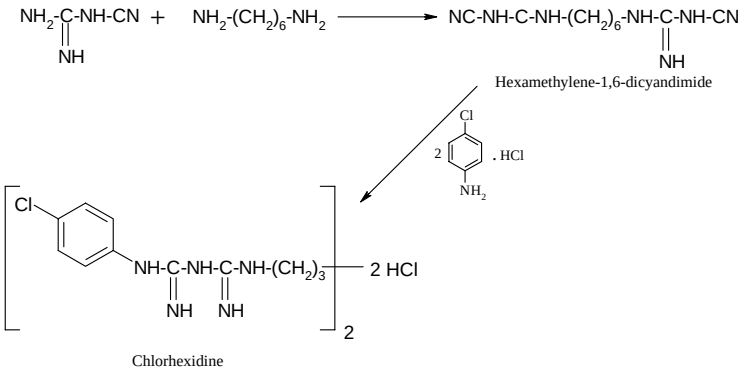
3. Synthesis of Hexyl resorcinol



Resorcinol

Hexyl resorcinol

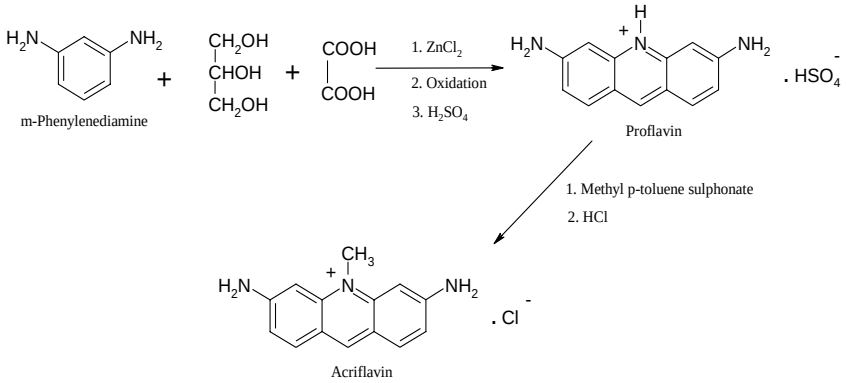
4. Synthesis of Chlorhexidine



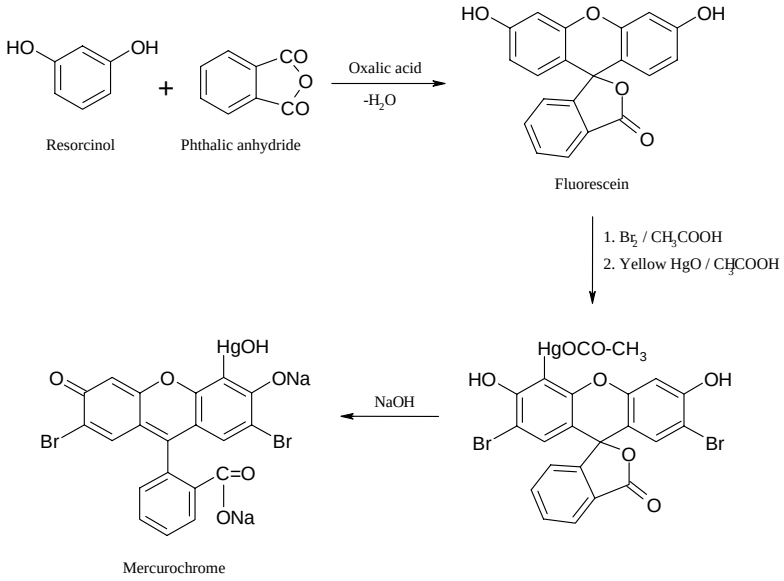
Hexamethylene-1,6-dicyandimide

Chlorhexidine

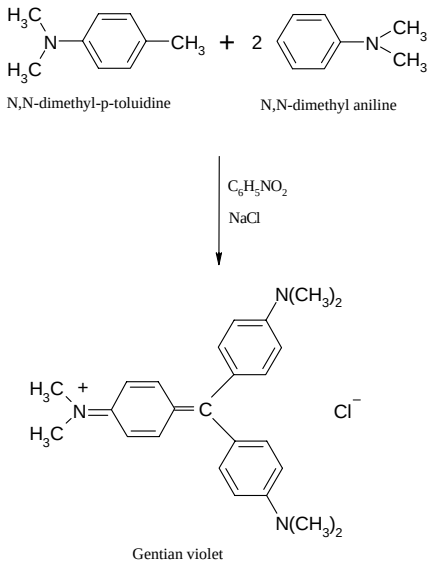
5. Synthesis of Acriflavin



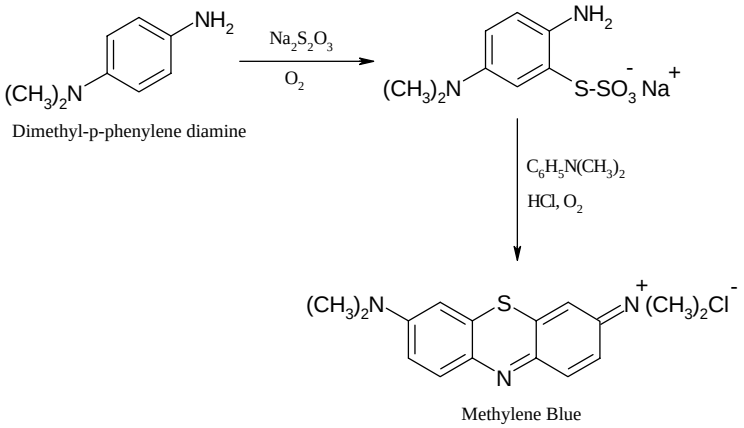
6. Synthesis of Mercurochrome



7. Synthesis of Gentian violet



8. Synthesis of Methylene Blue



Dimethyl-p-phenylene diamine

Methylene Blue

Mechanism of Action

Whatever the type of microbial cell (or entity), it is probable that there is a common sequence of events. This can be envisaged as interaction of the antiseptic or disinfectant with the cell surface followed by penetration into the cell and action at the target site(s). The nature and composition of the surface vary from one cell type (or entity) to another but can also alter as a result of changes in the environment. Interaction at the cell surface can produce a significant effect on viability (e.g. with glutaraldehyde), but most antimicrobial agents appear to be active intracellularly. The outermost layers of microbial cells can thus have a significant effect on their susceptibility (or insusceptibility) to antiseptics and disinfectants; it is disappointing how little is known about the passage of these antimicrobial agents into different types of microorganisms. Potentiation of activity of most biocides may be achieved by the use of various additives. The mechanisms of action are summarized in the table-2.

Table-2: Mechanisms of action of some antiseptics and disinfectants

Target	Antiseptic/ disinfectant	Mechanism of action
Cell envelope (cell wall, outer membrane)	Glutaraldehyde	Cross-linking of proteins
	EDTA, other permeabilizers	Gram-negative bacteria: removal of Mg^{2+}, release of some LPS
Cytoplasmic (inner) membrane	Chlorhexidine	Low concentrations affect membrane integrity, high concentrations cause congealing of cytoplasm
	Diamines	Induction of leakage of amino acids

	PHMB, alexidine	Phase separation and domain formation of membrane lipids
	Phenols	Leakage; some cause uncoupling
Cross-linking of macromolecules	Formaldehyde	Cross-linking of proteins, RNA, and DNA
	Glutaraldehyde	Cross-linking of proteins in cell envelope and elsewhere in the cell
DNA intercalation	Acridines	Intercalation of an acridine molecule between two layers of base pairs in DNA
Interaction with thiol groups	Silver compounds	Membrane-bound enzymes (interaction with thiol groups)
Effects on DNA	Halogens	Inhibition of DNA synthesis
	Hydrogen peroxide, silver ions	DNA strand breakage
Oxidizing agents	Halogens	Oxidation of thiol groups to disulfides, sulfoxides, or disulfoxides
	Peroxygens	Hydrogen peroxide: activity due to formation of free hydroxy radicals (·OH), which oxidize thiol groups in enzymes and proteins; PAA: disruption of thiol groups in proteins and enzymes

General Discussion about Some Important Antiseptics and Disinfectants

1. Alcohols

Ethanol : Erhanol is an effective antiseptic and can be used for a number of purposes in different concentrations. It can be used as antiseptic, preservative, mild counter-irritant solvent, astringent and rubefacient. It acts by precipitating the bacterial proteins. It as an irritant, and should not be applied to mucous membrane, delicate skin or open wound, as it may cause burning sensation. It is rarely used for disinfection as does not kills spores and may promote rusting. It is also used for the preparation of large number of pharmaceutical preparations like spirits and tinctures.

It occurs as a clear, colourless, volatile liquid, having a burning taste and a pleasant characteristic odour. It is soluble in water and most organic solvents. Ethanol is commercially prepared through fermentation process by the action of microbes on molases.

Isopropanol : Isopropanol has been found to be a suitable substitute for ethanol and is used to disinfect the skin and surgical instruments. It is more potent than ethanol. It cannot be used internally. It is used in a number of pharmaceutical and cosmetic preparations.

It occurs as a clear, colourless, volatile liquid, having a pleasant characteristic odour and a bitter taste. It is miscible with water, alcohol and chloroform.

2. Aldehydes

Glutaraldehyde : Glutaraldehyde is an important dialdehyde that has found usage as a disinfectant and sterilant, in particular for low-temperature disinfection and sterilization of endoscopes and surgical equipment and as a fixative in electron microscopy. Glutaraldehyde has a broad spectrum of activity against bacteria and their spores, fungi, and viruses, and a considerable amount of information is now available about the ways whereby these different organisms are inactivated. The mechanism of action is summarized in the table-3.

Table-3: Mechanism of antimicrobial action of Glutaraldehyde

Target microorganism	Glutaraldehyde action
Bacterial spores	Low concentrations inhibit germination; high concentrations are sporicidal, probably as a consequence of strong interaction with outer cell layers
Mycobacteria	Action unknown, but probably involves mycobacterial cell wall
Other non-sporulating bacteria	Strong association with outer layers of gram-positive and gram-negative bacteria; cross-linking of amino groups in protein; inhibition of transport processes into cell
Fungi	Fungal cell wall appears to be a primary target site, with postulated interaction with chitin
Viruses	Actual mechanisms unknown, but involve protein-DNA cross-links and capsid changes
Protozoa	Mechanism of action not known

Formaldehyde : Formaldehyde (methanal, CH_2O) is a monoaldehyde that exists as a freely water-soluble gas. Formaldehyde solution (formalin) is an aqueous solution containing *ca*. 34 to

38% (wt/wt) CH_2O with methanol to delay polymerization. Its clinical use is generally as a disinfectant and sterilant in liquid or in combination with low-temperature steam. Formaldehyde is bactericidal, sporicidal, and virucidal, but it works more slowly than glutaraldehyde.

Formaldehyde is an extremely reactive chemical that interacts with protein, DNA, and RNA *in vitro*. It has long been considered to be sporicidal by virtue of its ability to penetrate into the interior of bacterial spores. The interaction with protein results from a combination with the primary amide as well as with the amino groups, although phenol groups bind little formaldehyde. It has been proposed that formaldehyde acts as a mutagenic agent and as an alkylating agent by reaction with carboxyl, sulfhydryl, and hydroxyl groups. Formaldehyde also reacts extensively with nucleic acid (e.g., the DNA of bacteriophage T2). It forms protein-DNA cross-links in SV40, thereby inhibiting DNA synthesis. Low concentrations of formaldehyde are sporostatic and inhibit germination. Formaldehyde alters HBsAg and HBcAg of HBV.

It is difficult to pinpoint accurately the mechanism(s) responsible for formaldehyde-induced microbial inactivation. Clearly, its interactive, and cross-linking properties must play a considerable role in this activity. Most of the other aldehydes (glutaraldehyde, glyoxyl, succinaldehyde, and *o*-phthalaldehyde [OPA]) that have sporicidal activity are dialdehydes (and of these, glyoxyl and succinaldehyde are weakly active). The distance between the two aldehyde groups in glutaraldehyde (and possibly in OPA) may be optimal for interaction of these-CHO groups in nucleic acids and especially in proteins and enzymes.

Formaldehyde-releasing agents : Several formaldehyde-releasing agents have been used in the treatment of peritonitis. They include noxythiolin (oxymethylenethiourea), tauroline (a condensate of two molecules of the aminosulponic acid taurine with three molecules of formaldehyde), hexamine (hexamethylenetetramine, methenamine), the resins melamine and urea formaldehydes, and imidazolone derivatives such as dantoin. All of these agents are claimed to be microbicidal on account of the release of formaldehyde. However, because the antibacterial activity of taurolin is greater than that of free formaldehyde, the activity of taurolin is not entirely the result of formaldehyde action.

***o*-Phthalaldehyde:** OPA is a new type of disinfectant that is claimed to have potent bactericidal and sporicidal activity and has been suggested as a replacement for glutaraldehyde in endoscope disinfection. OPA is an aromatic compound with two aldehyde groups. To date, the mechanism of its antimicrobial action has been little studied, but preliminary evidence suggests an action similar to that of glutaraldehyde.

3. Anilides

The anilides have been investigated primarily for use as antiseptics, but they are rarely used in the clinic. Triclocarban (TCC; 3,4,4'-triclorocarbanilide) is the most extensively studied in this series and is used mostly in consumer soaps and deodorants. TCC is particularly active against Gram-positive bacteria but significantly less active against Gram-negative bacteria and fungi and lacks appreciable substantivity (persistency) for the skin. The anilides are thought to act by adsorbing to and destroying the semi permeable character of the cytoplasmic membrane, leading to cell death.

4. Biguanides

Chlorhexidine : Chlorhexidine is probably the most widely used biocide in antiseptic products, in particular in hand washing and oral products but also as a disinfectant and preservative. This is due in particular to its broad-spectrum efficacy, substantivity for the skin, and low irritation. Of note, irritability has been described and in many cases may be product specific. Despite the advantages of chlorhexidine, its activity is pH dependent and is greatly reduced in the presence of organic matter. A considerable amount of research has been undertaken on the mechanism of the antimicrobial action of this important bisbiguanide, although most of the attention has been devoted to the way in which it inactivates nonsporulating bacteria. Nevertheless, sufficient data are now available to examine its sporostatic and mycobacteriostatic action, its effects on yeasts and protozoa, and its antiviral activity. The mechanism of antimicrobial action is summarized in the table-4.

Table-4: Mechanism of antimicrobial action of Chlorhexidine

Type of microorganism	Chlorhexidine action
Bacterial spores	Not sporicidal but prevents development of spores; inhibits spore outgrowth but not germination.
Mycobacteria	Mycobacteristatic (mechanism unknown) but not mycobactericidal.
Other non-sporulating bacteria	Membrane-active agent, causing protoplast and spheroplast lyses; high concentrations cause precipitation of proteins and nucleic acids
Yeasts	Membrane-active agent, causing protoplast lysis and intracellular leakage; high concentrations cause intracellular coagulation.
Viruses	Low activity against many viruses; lipid-enveloped viruses more sensitive than nonenveloped viruses; effect possibly on viral envelope, perhaps the lipid moieties.
Protozoa	Recent studies against *A. castellanii* demonstrate membrane activity (leakage) toward trophozoites, less toward cysts.

Chlorhexidine is a bactericidal agent. Its interaction and uptake by bacteria were studied initially by Hugo et al., who found that the uptake of chlorhexidine by *E. coli* and *S. aureus* was very rapid and depended on the chlorhexidine concentration and pH. More recently, by using

[^{14}C]chlorhexidine gluconate, the uptake by bacteria and yeasts was shown to be extremely rapid, with a maximum effect occurring within 20 s. Damage to the outer cell layers takes place but is insufficient to induce lysis or cell death. The agent then crosses the cell wall or outer membrane, presumably by passive diffusion, and subsequently attacks the bacterial cytoplasmic or inner membrane or the yeast plasma membrane. In yeasts, chlorhexidine "partitions" into the cell wall, plasma membrane, and cytoplasm of cells. Damage to the delicate semipermeable membrane is followed by leakage of intracellular constituents, which can be measured by appropriate techniques. Leakage is not per se responsible for cellular inactivation but is a consequence of cell death. High concentrations of chlorhexidine cause coagulation of intracellular constituents. As a result, the cytoplasm becomes congealed, with a consequent reduction in leakage, so that there is a biphasic effect on membrane permeability. An initial high rate of leakage rises as the concentration of chlorhexidine increases, but leakage is reduced at higher biocide concentrations because of the coagulation of the cytosol.

Chlorhexidine was claimed by some scientists to be an inhibitor of both membrane-bound and soluble ATPase as well as of net K^+ uptake in *Enterococcus faecalis*. However, only high biguanide concentrations inhibit membrane-bound ATPase, which suggests that the enzyme is not a primary target for chlorhexidine action. Although chlorhexidine collapses the membrane potential, it is membrane disruption rather than ATPase inactivation that is associated with its lethal effects.

The effects of chlorhexidine on yeast cells are probably similar to those previously described for bacteria. Chlorhexidine has a biphasic effect on protoplast lysis, with reduced lysis at higher biguanide concentrations. Furthermore, in whole cells, the yeast cell wall may have some effect in limiting the uptake of the biguanide. The findings presented here and elsewhere demonstrate an effect on the fungal plasma membrane but with significant actions elsewhere in the cell. Increasing concentrations of chlorhexidine (up to 25 μg/ml) induce progressive lysis of *Saccharomyces cerevisiae* protoplasts, but higher biguanide concentrations result in reduced lysis.

Work to date suggests that chlorhexidine has a similar effect on the trophozoites of *Acanthameoba castellanii*, with the cysts being less sensitive. It has also been reviewed that chlorhexidine and other biocides affects *Acanthameoba* and the membrane damage in these protozoa is a significant factor in their inactivation.

Mycobacteria are generally highly resistant to chlorhexidine. Little is known about the uptake of chlorhexidine (and other antiseptics and disinfectants) by mycobacteria and on the biochemical changes that occur in the treated cells. Since the MICs for some mycobacteria are on the order of those for chlorhexidine-sensitive, gram-positive cocci, the inhibitory effects of chlorhexidine on mycobacteria may not be dissimilar to those on susceptible bacteria. *Mycobacterium avium-intracellulare* is considerably more resistant than other mycobacteria.

Chlorhexidine is not sporicidal. Even high concentrations of the bisbiguanide do not affect the viability of *Bacillus* spores at ambient temperatures, although a marked sporicidal effect is achieved at elevated temperatures. Presumably, sufficient changes occur in the spore structure to permit an increased uptake of the biguanide, although this has yet to be shown experimentally.

Little is known about the uptake of chlorhexidine by bacterial spores, although coatless forms take up more of the compound than do "normal" spores.

Chlorhexidine has little effect on the germination of bacterial spores but inhibits outgrowth. The reason for its lack of effect on the former process but its significant activity against the latter is unclear. It could, however, be reflected in the relative uptake of chlorhexidine, since germinating cells take up much less of the bisbiguanide than do outgrowing forms. Binding sites could thus be reduced in number or masked in germinating cells.

The antiviral activity of chlorhexidine is variable. Studies with different types of bacteriophages have shown that chlorhexidine has no effect on MS2 or K coliphages. High concentrations also failed to inactivate *Pseudomonas aeruginosa* phage F116 and had no effect on phage DNA within the capsid or on phage proteins; the transduction process was more sensitive to chlorhexidine and other biocides than was the phage itself. This substantiated an earlier finding that chlorhexidine bound poorly to F116 particles. Chlorhexidine is not always considered a particularly effective antiviral agent, and its activity is restricted to the lipid-enveloped viruses. Chlorhexidine does not inactivate nonenveloped viruses such as rotavirus, HAV, or poliovirus. Its activity was found to be restricted to the nucleic acid core or the outer coat, although it is likely that the latter would be a more important target site.

Alexidine : Alexidine differs chemically from chlorhexidine in possessing ethylhexyl end groups. Alexidine is more rapidly bactericidal and produces a significantly faster alteration in bactericidal permeability. Studies with mixed-lipid and pure phospholipid vesicles demonstrate that, unlike chlorhexidine, alexidine produces lipid phase separation and domain formation. It has been proposed that the nature of the ethylhexyl end group in alexidine, as opposed to the chlorophenol one in chlorhexidine, might influence the ability of a biguanide to produce lipid domains in the cytoplasmic membrane.

Polymeric biguanides: Vantocil is a heterodisperse mixture of polyhexamethylene biguanides (PHMB) with a molecular weight of approximately 3,000. Polymeric biguanides have found use as general disinfecting agents in the food industry and, very successfully, for the disinfection of swimming pools. Vantocil is active against Gram-positive and Gram-negative bacteria, although *P. aeruginosa* and *Proteus vulgaris* are less sensitive. Vantocil is not sporicidal. PHMB is a membrane-active agent that also impairs the integrity of the outer membrane of Gram-negative bacteria, although the membrane may also act as a permeability barrier. Activity of PHMB increases on a weight basis with increasing levels of polymerization, which has been linked to enhanced inner membrane perturbation.

Unlike chlorhexidine but similar to alexidine, PHMB causes domain formation of the acidic phospholipids of the cytoplasmic membrane. Permeability changes ensue, and there is believed to be an altered function of some membrane-associated enzymes. The proposed sequence of events during its interaction with the cell envelope of *E. coli* is as follows: (i) there is rapid attraction of PHMB towards the negatively charged bacterial cell surface, with strong and specific adsorption to phosphate-containing compounds; (ii) the integrity of the outer membrane is impaired, and PHMB is attracted to the inner membrane; (iii) binding of PHMB to phospholipids occurs, with an increase in inner membrane permeability (K^+ loss) accompanied by bacteriostasis; and (iv)

complete loss of membrane function follows, with precipitation of intracellular constituents and a bactericidal effect.

5. Diamidines

The diamidines are used for the topical treatment of wounds. The isothionate salts of two compounds, propamidine (4,4-diaminodiphenoxypropane) and dibromopropamidine (2,2-dibromo-4,4-diamidinodiphenoxypropane), have been used as antibacterial agents. The structure is given in the table-1.

The exact mechanism of action of diamidines is unknown, but they have been shown to inhibit oxygen uptake and induce leakage of amino acids (Table 2), as would be expected if they are considered as cationic surface-active agents.

6. Halogen-Releasing Agents

Halogens include iodine, chlorine, bromine, and fluorine. The disinfectant usually recommended for mold removal is a solution of one part bleach to two parts water. Commercial disinfectants are also available through janitorial supply stores. Use a household or garden sprayer and spray all surfaces that have been touched by flood water or have been soaked by water from some other source. Use a brush or broom to force the solution into crevices.

Iodine: Tincture of iodine (2% I_2 in 70% alcohol) inactivates proteins and is used as an antiseptic on skin. Iodine is one of the oldest (300 to 400 years) and most effective germicidal agents. It is a broad-spectrum bactericide and a good fungicide with some viricidal action. It will kill spores and is an excellent disinfectant that is effective against protozoa (amebas). It is only slightly soluble in water; iodine is available as a tincture dissolved in alcohol. Problems arise when the alcohol evaporates and the concentration of iodine increases, which can cause burning of skin.

Iodophors : Iodophors are combinations of iodine and organic molecules (hydrocarbons). Iodophors work by inhibiting enzyme action and are more effective than iodine. They are nonirritating, good surfactants, and non-staining.

Chlorine : Chlorine (Cl_2) gas forms hypochlorous acid (HClO), a strong oxidizing agent, and is used to disinfect drinking water and as a general disinfectant. Chlorine is used as a gas dissolved in water or in combination with other chemicals. The chlorine mode of operation is not completely understood but appears to be a strong oxidizing agent as result of the following reaction:

$$Cl_2 + H_2O \longrightarrow HCl + HClO \longrightarrow HCl + [O]$$

Hypochlorites are used domestically and industrially for disinfection. Chlorine is a universal disinfectant that is active against all microorganisms, including bacterial spores.

Potential applications for chlorine as a disinfectant include:

- Work surfaces
- Glassware
- Fixed or portable equipment and cages
- Liquids treated for discard

• Before and after vivarium entry, as a footbath

Many active chlorine compounds are available at various strengths; however, the most widely used for chemical disinfection is sodium hypochlorite. Household or laundry bleach is a solution of 5.25% (or 52,500 ppm) sodium hypochlorite. Note that a 10% or 1:10 dilution of bleach will result in a 0.525% or 5250-ppm solution of chlorine. The Centers for Disease Control and Prevention (CDC) recommends 500 ppm (1:100 dilution of household bleach) to 5000 ppm (1:10 dilution of bleach), depending on the amount of organic material present, to inactivate the human immunodeficiency virus (HIV). The strength of chlorine to be used for disinfection must be clearly indicated when described in standard operating procedures. Chlorine solutions will gradually lose strength, so fresh solutions must be prepared frequently. Diluted solutions should be replaced after 24 hours.

The stability of chlorine in solution is greatly affected by the following factors:
• Chlorine concentration
• Presence and concentration of catalysts such as copper or nickel
• pH of the solution
• Temperature of the solution
• Presence of organic material
• Ultraviolet irradiation

The chlorine solution should have the following characteristics for maximum stability:
• Low chlorine concentration
• Absence or low content of catalysts such as nickel or copper
• High alkalinity
• Low temperature
• Absence of organic materials

Chlorine should be shielded from ultraviolet light by storage in the dark in closed containers. The following factors may affect chlorine biocidal activity:

• **pH** — Chlorine is more effective at a lower pH.

• **Temperature** — An increase in temperature produces an increase in bactericidal activity.

• **Concentration** — A fourfold increase of chlorine will result in a 50% reduction in killing time, and a twofold increase results in a 30% reduction in killing time.

• **Organic material** — Organic material will consume available chlorine. If the organic material contains proteins, the reaction with chlorine will form chloramines that will have some antibacterial activity. Loss due to organic materials is more significant if minute amounts of chlorine are used. Footbaths are frequently contaminated with organic material and may require more frequent changing than the 24 hours previously stated.

• **Hardness** — Hardness of the water does not have a slowing effect on the antibacterial action of sodium hypochlorite.

• **Addition of ammonia or amino compounds** — Addition of ammonia and nitrogen compounds will slow the bactericidal action of chlorine.

Other available active chlorine sources include liquid chlorine, chlorine dioxide, inorganic chloramines, organic chloramines, and halazone.

Chlorine combines with protein and rapidly decreases in concentration when protein is present. This property gives rise to swimming pool odor which is often mistaken for the odor of chlorine. In actuality, that characteristic swimming pool odor indicates that the chlorine in the water has combined with organic contaminants and is off-gassing from the pool water. The organic source may be contamination in the pool (e.g., perspiration, urine, feces). Other natural non-protein materials and plastics and cationic detergents may also inactivate chlorine.

Chlorine is a strong oxidizing agent that is corrosive to metals and should not be used on the metal parts of machines that are subject to stress when in use. Do not autoclave chlorine solutions or materials treated with them, as the residual chlorine can vaporize resulting in an inhalation hazard. Do not use chlorine in combination with ammonia, acetylene, butadiene, butane, methane, propane or other petroleum gases, hydrogen, sodium carbide, benzene, finely divided metals, or turpentine. Chlorine may cause irritation to the eyes, skin, and lungs. Wear safety goggles, rubber gloves, aprons, or other protective clothing when handling undiluted solutions.

7. Heavy Metals

Heavy metals are the most ancient of antiseptics and disinfectants. Heavy metals were used by Egyptians, in the form of gold ointments and dust, and were often buried with the corpse or mummies to provide salves and ointments in the afterlife. Heavy metals have an oligodynamic (all encompassing) action and are extremely effective. They work because of the strong affinity of the metals to proteins. Metallic ions bind and adhere to the sulfhydryl groups in proteins, and enzymatic bindings are created. Stronger concentrations act as protein precipitants. Low concentrations have a subtle interference on the metabolism of the cell. Examples of heavy metal usage as disinfectants include the use of copper for ionizing water and to control algae. DaVinci and others added gold dust to ointments for wounds.

Mercuric chloride inactivates proteins by reacting with sulfide groups and is used as a disinfectant, although it occasionally is also used as an antiseptic on skin. Mercurials (inorganic mercury compounds) have a long history, with their heyday occurring during World War I. Mercurials were replaced by organic mercury compounds such as mercurochrome, methiolate, and metaphen. These compounds were used as skin antiseptics but their effects are reversed when they are washed off. Due to the toxic effects of mercury, these compounds are no longer recommended for first aid or skin disinfection.

Zinc used in combination with chlorine compounds as a mouthwash and in other combinations is an effective fungicide. Organometallics (organically activated metals such as heavy metals or organic radicals such as alcohol) are effective against Gram positive cocci, diphtheroids, sporeforming rods, tuberculosis, and similar organisms and may be effective against viruses. They are extremely effective against mycoses and have virtually no effectiveness against Gram-negative rods. Tributyltin is an example of an organometallic that also has deodorizing qualities.

In one form or another, silver and its compounds have long been used as antimicrobial agents. The most important silver compound currently in use is silver sulfadiazine (AgSD), although silver metal, silver acetate, silver nitrate, and silver protein, all have antimicrobial properties. In recent years, silver compounds have been used to prevent the infection of burns and some eye infections and to destroy warts.

Silver nitrate: The mechanism of the antimicrobial action of silver ions is closely related to their interaction with thiol (sulfydryl, -SH) groups, although other target sites remain a possibility. It has also been demonstrated that amino acids such as cysteine and other compounds such as sodium thioglycolate containing thiol groups neutralized the activity of silver nitrate against *P. aeruginosa*. By contrast, amino acids containing disulfide (SS) bonds, non-sulfur-containing amino acids, and sulfur-containing compounds such as cystathione, cysteic acid, L-methionine, taurine, sodium bisulfite, and sodium thiosulfate were all unable to neutralize Ag^+ activity. These and other findings imply that interaction of Ag^+ with thiol groups in enzymes and proteins plays an essential role in bacterial inactivation, although other cellular components may be involved. Hydrogen bonding, the effects of hydrogen bond-breaking agents, and the specificity of Ag^+ for thiol groups have been discussed by some co-workers. Virucidal properties might also be explained by binding to -SH groups.

It has also been proposed that silver salts and other heavy metals such as copper act by binding to key functional groups of fungal enzymes. Ag^+ causes the release of K^+ ions from microorganisms; the microbial plasma or cytoplasmic membrane, with which is associated many important enzymes, is an important target site for Ag^+ activity.

In addition to its effects on enzymes, Ag^+ produces other changes in microorganisms. Silver nitrate causes marked inhibition of growth of *Cryptococcus neoformans* and is deposited in the vacuole and cell wall as granules. Ag^+ inhibits cell division and damages the cell envelope and contents of *P. aeruginosa*. Bacterial cells increase in size, and the cytoplasmic membrane, cytoplasmic contents, and outer cell layers all exhibit structural abnormalities, although without any blebs (protuberances). Finally, the Ag^+ ion interacts with nucleic acids; it interacts preferentially with the bases in DNA rather than with the phosphate groups, although the significance of this in terms of its lethal action is unclear.

Silver sulfadiazine: AgSD is essentially a combination of two antibacterial agents, Ag^+ and sulfadiazine (SD). The question whether the antibacterial effect of AgSD arises predominantly from only one of the compounds or via a synergistic interaction has been posed repeatedly. AgSD has a broad spectrum of activity and, unlike silver nitrate, produces surface and membrane blebs in susceptible (but not resistant) bacteria. AgSD binds to cell components, including DNA. Based on a chemical analysis, a polymeric structure of AgSD was proposed that was composed of six silver atoms bonding to six SD molecules by linkage of the silver atoms to the nitrogens of the SD pyrimidine ring. Bacterial inhibition would then presumably be achieved when silver binds to sufficient base pairs in the DNA helix, thereby inhibiting transcription. Similarly, its antiphage properties have been ascribed to the fact that AgSD binds to phage DNA. Clearly, the precise mechanism of action of AgSD has yet to be solved.

8. Peroxygens

Hydrogen peroxide : Hydrogen peroxide (H_2O_2) is a widely used biocide for disinfection, sterilization, and antisepsis. It is a clear, colorless liquid that is commercially available in a variety of concentrations ranging from 3 to 90%. H_2O_2 is considered environmentally friendly, because it can rapidly degrade into the innocuous products water and oxygen. Although pure solutions are generally stable, most contain stabilizers to prevent decomposition. H_2O_2 demonstrates broad-spectrum efficacy against viruses, bacteria, yeasts, and bacterial spores. In general, greater activity is seen against gram-positive than gram-negative bacteria; however, the presence of catalase or other peroxidases in these organisms can increase tolerance in the presence of lower concentrations. Higher concentrations of H_2O_2 (10 to 30%) and longer contact times are required for sporicidal activity, although this activity is significantly increased in the gaseous phase. H_2O_2 acts as an oxidant by producing hydroxyl free radicals ($OH^{\bullet}$) which attack essential cell components, including lipids, proteins, and DNA. It has been proposed that exposed sulfhydryl groups and double bonds are particularly targeted.

Peracetic acid : Peracetic acid (PAA) (CH_3COOOH) is considered a more potent biocide than hydrogen peroxide, being sporicidal, bactericidal, virucidal, and fungicidal at low concentrations (<0.3%). PAA also decomposes to safe by-products (acetic acid and oxygen) but has the added advantages of being free from decomposition by peroxidases, unlike H_2O_2, and remaining active in the presence of organic loads. Its main application is as a low-temperature liquid sterilant for medical devices, flexible scopes, and hemodialyzers, but it is also used as an environmental surface sterilant.

Similar to H_2O_2, PAA probably denatures proteins and enzymes and increases cell wall permeability by disrupting sulfhydryl (-SH) and sulfur (S-S) bonds.

Ozone: Ozone generators sold as air cleaners intentionally produce the gas ozone. Ozone is a molecule composed of three atoms of oxygen. Two atoms of oxygen form the basic oxygen molecule — the oxygen we breathe that is essential to life. The third oxygen atom can detach from the ozone molecule and reattach to molecules of other substances, thereby altering their chemical composition. Ozone is a toxic gas with vastly different chemical and toxicological properties from oxygen. The same chemical properties that allow high concentrations of ozone to react with organic material outside the body give it the ability to react with similar organic materials that make up the body, with potentially harmful health consequences. Relatively low amounts can cause chest pain, coughing, shortness of breath, and, throat irritation. Ozone may also worsen chronic respiratory diseases such as asthma and compromise the ability of the body to fight respiratory infections. Whether in its pure form or mixed with other chemicals, ozone can be harmful to health. When inhaled, ozone can damage the lungs.

9. Phenols

Phenolic-type antimicrobial agents have long been used for their antiseptic, disinfectant, or preservative properties, depending on the compound. It has been known for many years that, although they have often been referred to as "general protoplasmic poisons," they have membrane-active properties which also contribute to their overall activity.

Phenol induces progressive leakage of intracellular constituents, including the release of K^+, the first index of membrane damage, and of radioactivity from ^{14}C-labeled *E. coli*. Some co-workers have demonstrated that low concentrations of phenols (0.032%, 320 µg/ml) and other (nonphenolic) agents lysed rapidly growing cultures of *E. coli*, staphylococci, and streptococci and concluded that autolytic enzymes were not involved. Some have even proposed that phenol acts only at the point of separation of pairs of daughter cells, with young bacterial cells being more sensitive than older cells to phenol.

It has been showed with chlorinated bis-phenol fenticlor that there was a close relationship between bactericidal activity and leakage of 260-nm-absorbing material (leakage being induced only by bactericidal concentrations). Fentichlor affected the metabolic activities of *S. aureus* and *E. coli* and produced a selective increase in permeability to protons with a consequent dissipation of the proton motive force (PMF) and an uncoupling of oxidative phosphorylation. Chlorocresol has a similar action. Coagulation of cytoplasmic constituents at higher phenol concentrations, which causes irreversible cellular damage, has been described.

The phenolics possess antifungal and antiviral properties. Their antifungal action probably involves damage to the plasma membrane, resulting in leakage of intracellular constituents. Phenol does not affect the transduction of *P. aeruginosa* PAO by bacteriophage F116, has no effect on phage DNA within the capsid, and has little effect on several of the phage band proteins unless treatments of 20 min or longer are used.

Phenol: It is used in dentistry as an analgesic, for dressing of small wounds. In solutions with glycerol, it is used as an antiseptic, and analgesic in mouth ulcers and tonsillitis.

Cresol: It is many times active than phenol and is less damaging to the tissues. It is used for disinfection of utensils, excretory fluids and for washing hands.

Resorcinol : It is less potent than phenol but is keratolytic and antipruritic. It is used for the treatment of various skin disorders like ringworm, eczema, psoriasis, dermatitis.

10. Bis-phenols

The bis-phenols are hydroxy-halogenated derivatives of two phenolic groups connected by various bridges. In general, they exhibit broad-spectrum efficacy but have little activity against *P. aeruginosa* and molds and are sporostatic toward bacterial spores. Triclosan and hexachlorophene are the most widely used biocides in this group, especially in antiseptic soaps and hand rinses. Both compounds have been shown to have cumulative and persistent effects on the skin.

Triclosan: Triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether; Irgasan DP 300) exhibits particular activity against Gram-positive bacteria. Its efficacy against Gram-negative bacteria and yeasts can be significantly enhanced by formulation effects. For example, triclosan in combination with EDTA caused increased permeability of the outer membrane. Reports have also suggested that in addition to its antibacterial properties, triclosan may have anti-inflammatory activity. The specific mode of action of triclosan is unknown, but it has been suggested that the primary effects are on the cytoplasmic membrane. In studies with *E. coli*,

triclosan at subinhibitory concentrations inhibited the uptake of essential nutrients, while higher, bactericidal concentrations resulted in the rapid release of cellular components and cell death. Studies with a divalent-ion-dependent *E. coli* triclosan mutant for which the triclosan MIC was 10-fold greater than that for a wild-type strain showed no significant differences in total envelope protein profiles but did show significant differences in envelope fatty acids. Specifically, a prominent 14:1 fatty acid was absent in the resistant strain, and there were minor differences in other fatty acid species. It was proposed that divalent ions and fatty acids may adsorb and limit the permeability of triclosan to its site of action. Minor changes in fatty acid profiles were recently found in both *E. coli* and *S. aureus* strains for which the triclosan MICs were elevated; however, the MBCs were not affected, suggesting, as for other phenols, that the cumulative effects on multiple targets contribute to the bactericidal activity.

Hexachlorophene: Hexachlorophene (hexachlorophane; 2,2'-dihydroxy-3,5,6,3',5',6'-hexachloro diphenylmethane) is another bis-phenol whose mode of action has been extensively studied. The primary action of hexachlorophene, based on studies with *Bacillus megatherium*, is to inhibit the membrane-bound part of the electron transport chain, and the other effects noted above are secondary ones that occur only at high concentrations. It induces leakage, causes protoplast lysis, and inhibits respiration. The threshold concentration for the bactericidal activity of hexachlorphene is 10 µg/ml (dry weight), but peak leakage occurs at concentrations higher than 50 µg/ml and cytological changes occur above 30 µg/ml. Furthermore, hexachlorophene is bactericidal at 0°C despite causing little leakage at this temperature. Despite the broad-spectrum efficacy of hexachlorophene, concerns about toxicity, in particular in neonates, have meant that its use in antiseptic products has been limited.

11. Halophenols

Chloroxylenol: Chloroxylenol (4-chloro-3,5-dimethylphenol; *p*-chloro-*m*-xylenol) is the key halophenol used in antiseptic or disinfectant formulations. Chloroxylenol is bactericidal, but *P. aeruginosa* and many molds are highly resistant. Surprisingly, its mechanism of action has been little studied despite its widespread use over many years. Because of its phenolic nature, it would be expected to have an effect on microbial membranes.

12. Quaternary Ammonium Compounds

Surface-active agents (surfactants) have two regions in their molecular structures, one a hydrocarbon, water-repellent (hydrophobic) group and the other a water-attracting (hydrophilic or polar) group. Depending on the basis of the charge or absence of ionization of the hydrophilic group, surfactants are classified into cationic, anionic, nonionic, and ampholytic (amphoteric) compounds. Of these, the cationic agents, as exemplified by quaternary ammonium compounds (QACs), are the most useful antiseptics and disinfectants. They are sometimes known as cationic detergents. QACs have been used for a variety of clinical purposes (e.g., preoperative disinfection of unbroken skin, application to mucous membranes, and disinfection of noncritical surfaces). In addition to having antimicrobial properties, QACs are also excellent for hard-surface cleaning and deodorization.

It has been known for many years that QACs are membrane-active agents (Table-2) (i.e., with a target site predominantly at the cytoplasmic (inner) membrane in bacteria or the plasma membrane in yeasts). It has been proposed that the following sequence of events occurs with

microorganisms exposed to cationic agents: (i) adsorption and penetration of the agent into the cell wall; (ii) reaction with the cytoplasmic membrane (lipid or protein) followed by membrane disorganization; (iii) leakage of intracellular low-molecular-weight material; (iv) degradation of proteins and nucleic acids; and (v) wall lysis caused by autolytic enzymes. There is thus a loss of structural organization and integrity of the cytoplasmic membrane in bacteria, together with other damaging effects to the bacterial cell.

Useful information about the selectivity of membrane action can be obtained by studying the effects of biocides on protoplasts and spheroplasts suspended in various solutes. QACs cause lysis of spheroplasts and protoplasts suspended in sucrose. The cationic agents react with phospholipid components in the cytoplasmic membrane, thereby producing membrane distortion and protoplast lysis under osmotic stress. Isolated membranes do not undergo disaggregation on exposure to QACs, because the membrane distortion is not sufficiently drastic. The non-QAC agents TCC and trichlorosalicylanide have specific effects: TCC induces protoplast lysis in ammonium chloride by increasing Cl^- permeability, whereas trichlorosalicylanide induces lysis in ammonium nitrate by increasing NO_3^- permeability. In contrast, QACs (and chlorhexidine) induce lysis of protoplasts or spheroplasts suspended in various solutes because they effect generalized, rather than specific, membrane damage.

The bacterial cytoplasmic membrane provides the mechanism whereby metabolism is linked to solute transport, flagellar movement, and the generation of ATP. Protons are extruded to the exterior of the bacterial cell during metabolism. The combined potential (concentration or osmotic effect of the proton and its electropositivity) is the PMF, which drives these ancillary activities. The QAC cetrimide was found to have an effect on the PMF in *S. aureus*. At its bacteriostatic concentration, cetrimide caused the discharge of the pH component of the PMF and also produced the maximum amount of 260-nm-absorbing material.

QACs are also believed to damage the outer membrane of gram-negative bacteria, thereby promoting their own uptake. This aspect of QACs is considered below (see "Intrinsic resistance of gram-negative bacteria").

The QAC cetylpyridium chloride (CPC) induces the leakage of K^+ and pentose material from the yeast *S. cerevisiae* and induces protoplast lysis as well as interacting with crude cell sap. Unlike chlorhexidine, however, no biphasic effect on protoplast lysis was observed. The initial toxic effect of QACs on yeast cells is a disorganization of the plasma membranes, with organized lipid structures in the membranes (and in lipid bilayers) being disrupted.

QACs are sporostatic; they inhibit the outgrowth of spores (the development of a vegetative cell from a germinated spore) but not the actual germination processes (development from dormancy to a metabolically active state), albeit by an unknown mechanism. Likewise, the QACs are not mycobactericidal but have a mycobacteriostatic action, although the actual effects on mycobacteria have been little studied.

The QACs have an effect on lipid, enveloped (including human immunodeficiency virus and HBV) but not nonenveloped viruses. QAC-based products induced disintegration and morphological changes of human HBV, resulting in loss of infectivity. In studies with different

phages, CPC significantly inhibited transduction by bacteriophage F116 and inactivated the phage particles. Furthermore, CPC altered the protein bands of F116 but did not affect the phage DNA within the capsid.

13.Dyes

Staining is the main problem associated with all dyes. Dyes are used primarily in selective and differential media and can be used intravenously and as pills or applied to the skin in liquid form. Some dyes may be strong mutagenic agents, and the actions of some are unclear. When used as gaseous chemosterilizers, these disinfectant aerosol particles should be between 1 and 5 µm in size to be most effective.

Gentian violet : Gentian violet is a Rosaline dye which is active against gram-positive bacteria, staphylococci, and fungi but not against mycobacterium and gram-negative bacteria. It is used for the treatment of tinea and yeast infections, ulcers, eczema, Vincent's angina, and vaginitis as suppository. It is also used as an anthelmintic in thread worm and ring worm infections.

It occurs as a green powder or green flakes, having a metallic lusture. It is soluble in water and alcohol but insoluble in most of the non-polar organic solvents.

Basic fuschin: Basic fuschin is also a Rosaline dye which is a mixture of chlorides of Rosaline and *p*-rosalines and is similar to gentian violet. It is used topically for the treatment of ring worm infections and athlete's foot.

It occurs as green crystalline powder having metallic lusture, soluble in water and alcohol but insoluble in ether.

Methylene blue: Methylene blue is a thiazine derivative dye possessing redox properties which makes it useful for the treatment of cyanide poisoning. It is considered to be bacterioststic and is used for the treatment of cystitis and urethritis.

It occurs as green crystalline powder having metallic lusture, and soluble in water and alcohol.

Acriflavin and Proflavin: Acriflavin and Proflavin are acridine dyes which are active against gram-positive bacteria and gonococci. They are the most useful antiseptics among the dyes. They are non-irritant and their activity is decreased in the presence of organic matter but increased in the presence of alkali. They are useful for application to wounds, cuts and ulcers.

They occur as orange-yellow crystalline powder.

Mercurochrome: Mercurochrome is a mercury derivative, used as an antiseptic but has weak bacteriostatic properties. it is non-irritating and is used topically on cuts, wounds, skin, and mucosa. Its staining properties restrict its use.

14. Furan derivative

Nitrofuran : Nitrofuran is a broad spectrum antiseptic that has activity against both gram-positive and gram-negative bacteria, but no activity against fungi. It is bactericidal against most

bacteria but *Pseudomonas aeruginosa* strains are resistant. Its activity is reduced in the presence of serum. It inhibits the necessary enzymes for carbohydrate metabolism in bacteria and thus exerts its action. It is generally used as creams, dusting powder or solutions in the treatment of burns and skin grafts, minor cuts, and wounds.

It occurs as lemon yellow crystalline powder that is very slightly soluble in water while insoluble in most organic solvents.

Factors modifying the action of Antiseptics and Disinfectants

1. Temperature
2. pH
3. Concentration of the compound
4. Surface tension
5. Time of contact with the microbes
6. Nature and amount of microbes
7. Pressure of organic matter and body fluids
8. Nature of the compound
9. Nature of the surface to which they are applied

Suggested Readings:

1. M.E. Wolf: Burger`s Medicinal Chemistry, John Wiley and Sons, New York.
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