Disinfectants
2nd prof Pharm D
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Disinfectants are those chemicals that destroy pathogenic bacteria from inanimate surfaces. Some chemicals when used at apropriate concentration for appropriate duration can be used for sterilization and are called sterilant liquids. Those chemicals that can be safely applied over skin and mucus membranes are called antiseptics.

An ideal antiseptic or disinfectant should have following properties:

1. Should have wide spectrum of activity
2. Should be able to destroy microbes within practical period of time
3. Should be active in the presence of organic matter
4. Should make effective contact and be wettable
5. Should be active in any pH
6. Should be stable
7. Should have long shelf life
8. Should be speedy
9. Should have high penetrating power
10. Should be non-toxic, non-allergenic, non-irritative or non-corrosive
11. Should not have bad odour
12. Should not leave non-volatile residue or stain
13. Efficacy should not be lost on reasonable dilution
14. Should not be expensive and must be available easily

Such an ideal disinfectant is not yet available. The level of disinfection achieved depends on contact time, temperature, type and concentration of the active ingredient, the presence of organic matter, the type and quantum of microbial load. The chemical disinfectants at working concentrations rapidly lose their strength on standing.

Classification of disinfectants:

1. Based on consistency
   (a) Liquid (E.g., Alcohols, Phenols)
   (b) Gaseous (Formaldehyde vapour)
2. Based on spectrum of activity
   (a) High level
   (b) Intermediate level

Notes

3. Based on mechanism of action
   (a) Action on membrane (E.g., Alcohol, detergent)
   (b) Denaturation of cellular proteins (E.g., Alcohol, Phenol)
   (c) Oxidation of essential sulphydryl groups of enzymes (E.g., H_2O_2, Halogens)
   (d) Alkylation of amino-, carboxyl- and hydroxyl group (E.g., Formaldehyde)
   (e) Damage to nucleic acids (Formaldehyde)

Alcohols

Mode of action: Alcohols dehydrate cells, disrupt membranes and cause coagulation of protein.

Examples: Ethyl alcohol, isopropyl alcohol and methyl alcohol

Application: A 70% aqueous solution is more effective at killing microbes than absolute alcohols. 70% ethyl alcohol (spirit) is used as antiseptic on skin. Isopropyl alcohol is preferred to ethanol. It can also be used to disinfect surfaces. It is used to disinfect clinical thermometers. Methyl alcohol kills fungal spores, hence is useful in disinfecting inoculation hoods.

Disadvantages: Skin irritant, volatile (evaporates rapidly), inflammable

Aldehydes

Mode of action: Acts through alkylation of amino-, carboxyl- or hydroxyl group, and probably damages nucleicacids. It kills all microorganisms, including spores.

Examples: Formaldehyde, Gluteraldehyde

Application: 40% Formaldehyde (formalin) is used for surface disinfection and fumigation of rooms, chambers, operation theatres, biological safety cabinets, wards, sick rooms etc. Fumigation is achieved by boiling formalin, heating paraformaldehyde or treating formalin with potassium permanganate. It also sterilizes bedding, furniture and books. 10% formalin with 0.5% tetraborate sterilizes clean metal instruments. 2% gluteraldehyde is used to sterilize thermometers, cystoscopes, bronchoscopes, centrifuges, anasethetic equipments etc. An exposure of at least 3 hours at alkaline pH is required for action by gluteraldehyde. 2% formaldehyde at 40°C for 20 minutes is used to disinfect wool and 0.25% at 60^{O}C for six hours to disinfect animal hair and bristles.

Disadvantages: Vapors are irritating (must be neutralized by ammonia), has poor penetration, leaves non-volatile residue, activity is reduced in the presence of protein. Gluteraldehyde requires alkaline pH and only those articles that are wettable can be sterilized.

Phenol

Mode of action: Act by disruption of membranes, precipitation of proteins and inactivation of enzymes.

Examples: 5% phenol, 1-5% Cresol, 5% Lysol (a saponified cresol), hexachlorophene, chlorhexidine, chloroxylenol (Dettol)

Applications: Joseph Lister used it to prevent infection of surgical wounds. Phenols are coal-tar derivatives. They act as disinfectants at high concentration and as antiseptics at low concentrations. They are bactericidal, fungicidal, mycobactericidal but are inactive against spores and most viruses. They are not readily inactivated by organic matter. The corrosive phenolics are used for disinfection of ward floors, in discarding jars in laboratories and disinfection of bedpans. Chlorhexidine can be used in an isopropanol solution for skin disinfection, or as an aqueous solution for wound irrigation. It is often used as an antiseptic hand wash. 20% Chlorhexidine gluconate solution is used for pre-operative hand and skin preparation and for general skin disinfection. Chlorhexidine gluconate is also mixed with quaternary ammonium compounds such as cetrimide to get stronger and broader antimicrobial effects (eg. Savlon). Chloroxylenols are less irritant and can be used for topical purposes and are more effective against gram positive bacteria than gram negative bacteria. Hexachlorophene is chlorinated diphenyl and is much less irritant. It has marked effect over gram positive bacteria but poor effect over gram negative bacteria, mycobacteria, fungi and viruses. Triclosan is an organic phenyl ether with good activity against gram positive bacteria and effective to some extent against many gram negative bacteria including Pseudomonas. It also has fair activity on fungi and viruses.

Disadvantages: It is toxic, corrosive and skin irritant. Chlorhexidine is inactivated by anionic soaps. Chloroxylenol is inactivated by hard water.

Halogens

Mode of action: They are oxidizing agents and cause damage by oxidation of essential sulfydryl groups of enzymes. Chlorine reacts with water to form hypochlorous acid, which is microbicidal.

Examples: Chlorine compounds (chlorine, bleach, hypochlorite) and iodine compounds (tincture iodine, iodophores)

Applications: Tincture of iodine (2% iodine in 70% alcohol) is an antiseptic. Iodine can be combined with neutral carrier polymers such as polyvinylpyrrolidone to prepare iodophores such as povidone-iodine. Iodophores permit slow release and reduce the irritation of the antiseptic. For hand washing iodophores are diluted in 50% alcohol. 10% Povidone Iodine is used undiluted in pre and postoperative skin disinfection. Chlorine gas is used to bleach water. Household bleach can be used to disinfect floors. Household bleach used in a stock dilution of 1:10. In higher concentrations chlorine is used to disinfect swimming pools. 0.5% sodium hypochlorite is used in serology and virology. Used at a dilution of 1:10 in decontamination of spillage of infectious material. Mercuric chloride is used as a disinfectant.

Disadvantages: They are rapidly inactivated in the presence of organic matter. Iodine is corrosive and staining. Bleach solution is corrosive and will corrode stainless steel surfaces.

Heavy Metals

Mode of action: Act by precipitation of proteins and oxidation of sulfydryl groups. They are bacteriostatic.

Examples: Mercuric chloride, silver nitrate, copper sulfate, organic mercury salts (e.g., mercurochrome, merthiolate)

Applications: 1% silver nitrate solution can be applied on eyes as treatment for opthalmia neonatorum (Crede's method). This procedure is no longer followed. Silver sulphadiazine is used topically to help to prevent colonization and infection of burn tissues. Mercurials are active against viruses at dilution of 1:500 to 1:1000. Merthiolate at a concentration of 1:10000 is used in preservation of serum. Copper salts are used as a fungicide.

Disadvantages: Mercuric chloride is highly toxic, are readily inactivated by organic matter.

Surface Active Agents

Mode of actions: They have the property of concentrating at interfaces between lipid containing membrane of bacterial cell and surrounding aqueous medium. These compounds have long chain hydrocarbons that are fat soluble and charged ions that are water-soluble. Since they contain both of these, they concentrate on the surface of membranes. They disrupt membrane resulting in leakage of cell constituents.

Examples: These are soaps or detergents. Detergents can be anionic or cationic. Detergents containing negatively charged long chain hydrocarbon are called anionic detergents. These include soaps and bile salts. If the fat-soluble part is made to have a positive charge by combining with a quaternary nitrogen atom, it is called cationic detergents. Cationic detergents are known as quaternary ammonium compounds (or quat). Cetrimide and benzalkonium chloride act as cationic detergents.

Application: They are active against vegetative cells, Mycobacteria and enveloped viruses. They are widely used as disinfectants at dilution of 1-2% for domestic use and in hospitals.

Disadvantages: Their activity is reduced by hard water, anionic detergents and organic matter. Pseudomonas can metabolise cetrimide, using them as a carbon, nitrogen and energy source.

Dyes

Mode of action: Acridine dyes are bactericidal because of their interaction with bacterial nucleic acids.

Examples: Aniline dyes such as crystal violet, malachite green and brilliant green. Acridine dyes such as acriflavin and aminacrine. Acriflavine is a mixture of proflavine and euflavine. Only euflavine has effective antimicrobial properties. They are more effective against gram positive bacteria than gram negative bacteria and are more bacteriostatic in action.

Applications: They may be used topically as antiseptics to treat mild burns. They are used as paint on the skin to treat bacterial skin infections. Melachite green is used in LJ medium for growth of Mycobacterium *tuberculosis*.

Hydrogen Peroxide

Mode of action: It acts on the microorganisms through its release of nascent oxygen. Hydrogen peroxide produces hydroxyl-free radical that damages proteins and DNA.

Application: It is used at 6% concentration to decontaminate the instruments, equipments such as ventilators. 3% Hydrogen Peroxide Solution is used for skin disinfection and deodorising wounds and ulcers. Strong solutions are sporicidal.

Disadvantages: Decomposes in light, broken down by catalase, proteinaceous organic matter drastically reduces its activity.

Beta-propiolactone (BPL)

Mode of action: It is an alkylating agent and acts through alkylation of carboxyl-and hydroxyl-groups.

Properties: It is a colorless liquid with pungent to slightly sweetish smell. It is a condensation product of ketane with formaldehyde.

Application: It is an effective sporicidal agent, and has broad-spectrum activity. 0.2% is used to sterilize biological products. It is more efficient in fumigation that formaldehyde. It is used to sterilize vaccines, tissue grafts, surgical instruments and enzymes

Disadvantages: It has poor penetrating power and is a carcinogen.

Testing of Disinfectants

A disinfectant must be tested to know the required effective dilution, the time taken to effect disinfection and to periodically monitor its activity. As disinfectants are known to lose their activity on standing as well as in the presence of organic matter, their activity must be periodically tested.

Different methods are:

1. Koch's method
2. Rideal Walker Method
3. Chick Martin test
4. Capacity use dilution test (Kelsey-Sykes test)
5. In-use test

Koch's method: Spores of *Bacillus anthracis* were dried on silk thread and were subjected to action of disinfectants. Later, it was washed and transferred to solid medium.

Rideal Walker method: This method relies on the estimation of phenol coefficient. Phenol coefficient of a disinfectant is calculated by dividing the dilution of test disinfectant by the dilution of phenol that disinfects under predetermined conditions. Disadvantages of the Rideal-Walker test are: No organic matter is included; the microorganism Salmonella typhi may not be appropriate; the time allowed for disinfection is short; it should be used to evaluate phenolic type disinfectants only.

Chick Martin test: This test also determines the phenol coefficient of the test disinfectant. Unlike in Rideal Walker method where the test is carried out in water, the disinfectants are made to act in the presence of yeast suspension (or 3% dried human feces). Time for subculture is fixed at 30 minutes and the organism used to test efficacy is *S.typhi* as well as *S.aureus*. The phenol coefficient is lower than that given by Rideal Walker method.

Capacity use dilution test (Kelsey-Sykes test)

The capacity test (Kelsey-Sykes) determine the appropriate use dilution of the disinfectants. The stability test (Maurer) determines the stability and long term effectiveness of disinfectant dilution. The capacity and stability test help to determine the choice of a disinfectant.

In-use test:

The routine monitoring of disinfectant in use can be done by the ‘in use’ test (Kelsey & Maurer). This test is intended to estimate the number of living organism in a vessel of disinfectant in actual use. The disinfectant that is already in use is diluted 1 in 10 by mixing 1 ml of the disinfectant with 9 ml of sterile nutrient broth. Ten drops of the diluted disinfectant (each 0.02 ml) is placed on two nutrient agar plates. One plate is incubated at 37°C for 3 days while the other is held at room temperature for 7 days. The number of drops that yielded growth is counted after incubation. If there growth in more than five drops on either plate, it represents failure of disinfectant.