DIGITAL NOTE

Topic: Disinfection

Subject: Pharmaceutical Microbiology
Prepared By: Dr Sanjeshkumar G Rathi
Institute: Saraswati Institute of Pharmaceutical Sciences

Chapter: DISINFECTION

Definition:

- "Disinfection is the process of destruction or removal of micro-organisms & reducing them to a level not harmful to health."

Difference between Disinfection & Antiseptic:

- Disinfection: If the object is lifeless such as working area, dishes, bench etc; the chemical agent is known as disinfectant.
- Antiseptic: If the object is live, such as a human body tissue, the chemical is known as an antiseptic.
- Disinfectants are usually bactericidal but occasionally they may be bacteriostatic.
- "Iodine" is the chemical that is used as disinfectant as well as an antiseptic.
- Aseptic: Aseptic means free of contaminating micro-organisms.

Ideal Properties of Disinfectants:

- Broad spectrum
- Nontoxic
- Fast acting
- Odorless
- Surface compatibility
- Economical
- Easy to use
- Solubility & miscibility
- Not affected by physical factors
- Stable on storage

Classification of Disinfectants:

1. Acids & alkalis
2. Heavy metals
3. Alcohols
4. Quaternary ammonium compounds
5. Halogens
6. Phenol & its derivatives
7. Aldehydes
8. Dyes
9. Detergents & soaps

Classification of Disinfectants:

1. **Acids & Alkalis:**
   - Germicidal efficiency of acids & concentration of H^+ ions & their solutions.
   - E.g.:
   - (1) Mineral acids:HCl,H2SO4,HNO3,H3PO4
   - (2) Acids which are weakly ionized like benzoic acid, salicylic acid & acetic acid.

- These acids produce H+ ions conc. but also produce toxic effect because of additional effect of anions or to the undissociated molecules.
- Germicidal action of Alkalis is dependent on dissociation & release of OH-ions in solution.
- Note: Strong acids & alkalis are not especially useful because they are corrosive.

2. Halogens:

- e.g.-Chlorine, Iodine & fluoride
- Br & fluoride are irritant & difficult to handle; but Cl & Br are commonly used for sterilization.
- Cl is available in organic, inorganic & gaseous forms, mainly used to keep bacterial population at low level in municipal water.

Mechanism of Action:

- The germicidal action of Cl & its compounds is due to the formation of hypoclouras acid, when Cl reacts with water.
- Cl2 + H2O → HCl + HClO
- Hypochlorus Acid
- The hypochlorous acid is further decomposed into oxygen.
- 2HClO → 2HCl + O2
- The free oxygen released from this reaction is a strong oxidizing agent.
- It acts oncellular constituents of micro-organisms.
- Calcium hypochlorite [Ca(OCl)2] & sodium hypochlorite (NaOCl) are very widely used for disinfection of dairy equipment & eating utensils in restaurant.
- Chlorine compounds have been used to disinfect open wounds, to treat athletes' foot.
- Iodine is a very good disinfectant in aqueous or alcoholic solution.
- It is sporicidal, fungicidal & active against many viruses.

Uses:

- As a skin disinfectant & for cold sterilization of surgical sutures.
- E.g. -weak iodine solution B.P.
- Aqueous iodine solution B.P.

Mechanism of Action:

- Iodine is an oxidizing agent & it inactivates essential metabolic compounds such as protein with sulfhydral groups.

3. Heavy Metals:

- E.g. -Hg, Ag & Cu\

Mechanism of Action:

$$HS - Enzyme - SH + HgCl2 \rightarrow S - Enzyme - S + 2HCl$$

- Where S = Heavy metal like Hg
- High concentration of salts of heavy metals like Hg, cu & Ag coagulate cytoplasmicproteins, resulting in damage or death of the cell.

4. Phenol & its compounds:

- Phenol or carbolic acid is used as microbicidal agent & as a std. in evaluation of other disinfectants.

Mechanism of Action:

- Phenolic compounds mainly destroy the micro-organisms by the process of disruption of cell, precipitation of cell protein, inactivation of enzymes & leakage of amino acids from the cells.
- E.g. -o-cresol, p-cresol, chlorocresoletc.

5. **Alcohol:**

Mechanism of Action:

- They are protein denaturants.
- They may damage lipid complexes in the cell membrane.
- They are also dehydrating agents.
- Uses: 60-70% v/v alcohol uses as bactericidal action.
- Ethanol (60-70% v/v) & isopropanol (50-60% v/v) are used as skin disinfectants.
- Methanol vapour used as fungicide.
- 60% v/v concentration is effective against the viruses.
- CH3OH-Methanol, C2H5OH-Ethanol, (CH3)2CHOH-Isopropanol

6. **Aldehydes:**

- E.g. Formaldehyde (HCHO) is the main aldehyde used for disinfection.
- It is effective as a gas or as aqueous solution containing 34-38% w/w of HCHO.
- Aldehyde used to sterilize the certain medical instruments.

7. **<u>Quaternary Ammonium Compounds:</u>**

- E.g. Most of the cationic detergents are quaternary ammonium compounds.

Mechanism of Action:

- They are active against Gram positive bacteria & are also quite active against Gram – ve bacteria.
- The primary mechanism of action is disruption of cell walls & membrane.
- They also inactivate enzymes & denature proteins.
- E.g. Cetrimide, Benzalkonium chloride

Uses:

For control of micro-organisms on floors, walls, nursing homes & other public places.
Also used as skin antiseptics & as sanitizing agents in the dairy, egg & fishing industries.

8. **<u>Dyes:</u>**

- Basic dyes are more effective bactericides than acidic dyes.
- E.g. Acridine & trithenylmethome dyes
- They are more active against Gram positive bacteria than Gram negative bacteria.

Mechanism of Action:

- They impair the DNA complex of the micro-organisms thus kill or destroy the reproductive capacity of the cell.
- **Uses:** Treatment of burns, ophthalmic applications & bladder irrigation.

9. **<u>Detergents & Soaps:</u>**

They are classified into 4 main groups:

- Anionic
- Cationic
- Nonionic
- Amphoteric

- The most important antimicrobial agents are the "Cationic surface active agents".
- E.g. Cetrimide, Benzalkonium chloride
- Anionic compounds are like soap & SLS.
- Soaps prepared from "saturated fatty acids" are more effective against Gram negative bacilli.
- While those prepared from "unsaturated acids" have greater action against Gram positive & Neisseria group organism.

FACTORS AFFECTING DISINFECTANTS' ACTION:

1. Concentration of disinfectant
2. Temperature
3. Time of contact
4. pH of the environment
5. Surface tension
6. Formulation of the disinfectant
7. Chemical structure of disinfectant
8. Type & number of micro-organisms present
9. Interfering substances in the environment
10. Potentiating, synergism & antagonism of disinfectants

Concentration of Disinfectants:

- The rate of killing of micro-organisms varies directly with the concentration of the disinfectant.
- The effectiveness is generally related to concentration exponentially, not linearly.
- E.g. the optimum concentration of phenol at about 1% beyond this concentration, the disinfectant becomes less effective.
- The dilution co-efficient can be calculated from the following equation:

$$n = \log t2 - \log t1 / \log c1 - \log c2$$

- where,
- n=Dilution co-efficient of disinfectant
- t_1=Death time with disinfectant concentration c_1
- t_2=Death time with disinfectant concentration c_2

Temperature:

- The rate of disinfection normally increases with the temperature.
- The effect of temperature on bactericidal action may be expressed quantitatively by means of a temperature co-efficient (Θ).
- E.g. Per 10^o C rise in temperature is expressed by Θ^{10} or Q_{10} values.

$$\Theta^{10} \text{ or } Q_{10} = \frac{\text{Time required to kill at } T^o}{\text{Time required to kill at } (T+10)^o}$$

$$\Theta^{(T_2-T_1)} = t_1/t_2$$

- Where,
- T_2 & T_1=Temperature differing by 10^o C
- t_2 & t_1=Corresponding lethal time
- E.g. Q_{10} value for phenol=4 that means, the Q_{10} activity will be increased by a faster of 4.

Time of contact:

- Sufficient time of contact must be allowed for disinfectant to exert its action which shows that the principle of first order kinetics.

$$K = (1/t) * \log N_0/N_t$$

- Where,
- K = Efficiency of the disinfectant

pH of the environment:

- A pH range 6-8 is optimal for growth of many bacteria & the rate of growth declines on either side of this range. e.g.:
- Phenolic & acidic antimicrobial agent usually have greatest activity in acidic condition.
- Acridine dyes & quaternary ammonium compounds are more usually more active in alkaline than acidic solutions.

Surface Tension:

- If the disinfectant has surfactant property, then it helps in adsorption of surface active disinfectant on the surface of cell as well as in wetting and spreading property of solution.
- E.g: A combination of soap with crude phenol (carboxylic acid) has excellent disinfecting property.

Formulation of disinfectants:

- For effective use of disinfectant, its formulation is more important.
- e.g.: Effectiveness of chlorhexidine & quaternary ammonium compounds may be greater in 70% alcohol than in aqueous solution.
- For convenience & economy it is essential to prepare disinfectants as possible as concentrated & suitable for dilution with water immediately before use.

Chemical structure of disinfectants:

- Chemical structure of a compound affects the disinfectant activity.
- e.g.: Halogenations increases the antibacterial activity of phenol but nitration increases antibacterial activity & systemic toxicity also.

Types & numbers of microorganism present:

- The efficiency of disinfectant is greatly depends on nature & number of contaminating microorganisms & especially on the presence or absence of bacterial spores. e.g.:
- Aldehydes & halogens are effective against virus.
- Mycobacterium tuberculosis and other acid fast bacilli are fairly resistant to manu aqueous bactericides but are succeptible to iodine, formaldehyde & alcohol & phenolic compound.

Interfering substances in environment

- Blood, body fluids, pus, milk , food rsidue, or proteins may reduce the effectiveness of disinfectants is present in small amount.
- It may due to chemical reaction or absorption or shielding of m/o from adequate contact with germicide.
- The presence of oils and fat markedly reduce the disinfectant ability of phenolics.

Potentiation & synergism & antagonism of disinfectant:

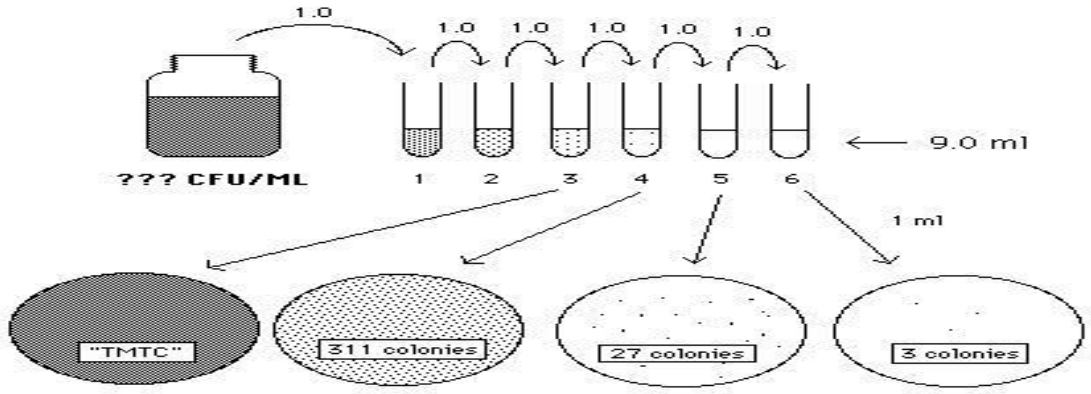
- Potentiation leads to enhanced antimicrobial activity
- e.g.: polysorbate 80, low concentration of
- Synergism effect of two antibiotics increases the antibiotic effect
- e.g.: Different p- hydroxybenzoate esters
- Antagonism leads to decrease antibacterial effect.
- e.g.: Na-thiosulphate

EVALUATION OF ANTIBACTERIAL AGENTS & DISINFECTANTS

1. Tube dilution & agar plate method
2. Filter paper & cup plate method
3. Ditch plate method
4. Phenol coefficient method
5. Kelsey sykes method

Tube dilution & agar plate method

- The chemical agent is incorporated into nutrient broth or agar media & incubated with m/o.
- These tubes are incubated at 30 to 37^0 C for 2-3 days & then observed the results in the form of "Turbidity" or "Colonies".
- Results were recorded & activity compared to given disinfectant as shown following



Filter paper, Cup plate & cylinder plate method

- In this method the agar is melted , cooled at 45 C , inoculated with the test micro organism & poured into a sterile Petri dish.
- When the inoculated agar has solidified, holes about 9 mm in diameter are cut in the medium with the sterile bore.
- The antibiotic is placed directly in the hole.
- The zone of inhibition is observed after incubation at 30-35 C for 2-3 days.
- The diameter of zone of inhibition giving an indication of the relative activities of different antibacterial agents against the test micro organism.

Ditch plate method

- The solution of anti microbial agent is carefully run into the ditch which is prepared in agar plate. The loop full of each test m/o. is then streaked outward from the ditch on the agar surface.
- The zone of inhibition found adjacent to the ditch or centre of plate.
- The width of inhibition zone gives an indication of the relative activity of the antibacterial agent against the various test m/o.

Phenol co-efficient method

- Phenol is used as reference standard to find out the antibacterial activity of test disinfectants. This test includes:

- Rideal-walker test (RW test)
- Chick-martin test(CM)
- United state Food and Drud Administration test(FDA test)
- The US Association of Official Agricultural Chemist test (AOAC test)

Rideal-walker test (RW test)

- Phenol co-efficient of the test disinfectant may be calculated by "Rideal-Walker test" that RW broth & S.typhy as sensitive m/o.
- % ml of each dilution are inoculated with 0.5 ml of the 24 hrs. broth culture of the m/o.
- All tubes (Disinfectant + organism and phenol + organism) are placed in a 17.5 C water bath.
- After 2.5, 5, 7.5 & 10 minutes time interval, subculture of each reaction mixture are taken & transferred into 5 ml sterile broth.
- The broth are incubated at 37 C for 48-72 hrs. & are examined for the presence or absence of growth.

Determination of Rideal-Walker coefficient

Disinfectant	**Dilution**	**Time Required for subculture (min)**			
		2.5	**5**	**7.5**	**10**
Test Disinfectant	1:1000	+	-	-	-
	1:2000	+	+	-	-
	1:3000	+	+	+	-
	1:4000	+	+	+	+
Phenol	1:80	+	-	-	-
	1:100	+	+	-	-
	1:120	+	+	+	-

	1:140	+	+	+	+
(note : + = growth, - = No growth)					

RW co-efficient = $\frac{\text{Dilution of disinfectant killing in 7.5 min but not in 5 min}}{\text{Dilution of Phenol killing in 7.5 min but not in 5 min}}$

= 2000/100

= 20

Inference:

- A phenolic co-efficient or RW co-efficient of given test disinfectant is 1 means that disinfectant has the same effectiveness as phenol.
- Phenol coefficient of test disinfectant is less than 1, that means it is less effectiveness & if more than 1 means it is more effective.
- If it is 20 means disinfectant is 20 times more active than phenol.

Points of difference	**RW Test**	**FDA Test**	**CM Test**	**AOAC Test**
Ph medium	7.4	6.8	7.4	6.8
Volume of medium	5.0	10.0	10.0	10.0
Volume of reaction medium	5.0	10.0	5.0	5.0
Diluents	water	water	Yeast suspension	water
Reaction temperature(C)	17/18	20	30	20/37
Test m/o.	S.Typhy	S.Typhy	S.Typhy	S.Typhy S.Aureus
Sampling time(min)	2.5,5,7.5,10	5,10,15	30	5,10
Calculation of phenol	Dilution of disinfectant	Dilution of disinfectant	Mean of highest phenol	Greatest dilution of test
coefficient	killing in 7.5 min but not in 5 min divided by that of phenol	killing in 10 min but not in5 min divided by that of phenol	Conc. Inhibiting & lowest permitting growth divided by same for test	killing in 10 min divided by same for phenol

Advantages of Phenol co-efficient method:

- Inexpensive & quickly performed.
- Reproducible result.

- Supply standards for crude preparations.

Disadvantages of Phenol co-efficient method :

- Choice of the test organism –In most of test only one m/o. is used (S.Typhy) therefore result for this organism gives only limited information on how the disinfectant will behave against other m/o.
- The test compares the activity of bactericides at only one concentration with a fixed death time & reaction temperature.
- It gives no indication of the activity of disinfectant in presence of organic matter.
- No information regarding tissue toxicity.
- Sampling errors.
- Not gives any indication of the effects of dilution on the activity of the disinfectant.
- Only used to evaluate phenolic disinfectant only.