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A Review Article On *Nirjivanukaran* W.S.R To Sterilization

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ABSTRACT: The term “sterilization” means removal or killing of microorganisms from the surface of the object. In *Ayurveda* Sterilization is also known as “*Nirjivanukaran*”. The *Acharya*’s mentioned thousands year back that the *Nirjivanukaran* is important before commencement of any surgical procedure. The different methods of sterilizations adopted by *Acharya*’s were *Dhupana Karama*, *Vrana Prakshalna*, *Shastra Tapan*, sterilization of *Kumaragara*, *Sootikagaara*, *Vranitagaara*, water filtration methods like *Hansodaka*, and use of flowers in water purification. The sterilization is done to control infection, sometimes infection spreads in large scale. In history we have many such examples which shows death of population on large scale due to infection i.e. plaque. The disease like plaque killed approximately 75-200 million of people with no cure. The infection claims millions of death every year which can be prevented by adopting proper sterilization methods. In 21st century modern science led to invention of many vaccines, preservatives, chemical formulations, newer techniques of air fumigation, sterilization by various physical & chemical methods and water filtration methods. In this article we have discussed many such methods. In this article attempt is made to know ancient techniques of sterilization in *Ayurveda* and to understand basic concepts & proper techniques of existing sterilization methods.

Key words: *Nirjivanukaran*, *Sterilization*, *Infection*

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INTRODUCTION

The process of evolution took millions of years to develop human and other species. With the evolution of human beings the diseases also started propagating at the same pace because of biological evolution of organisms, the man no longer remained free from diseases. The evolution undergone many natural changes to adopt the existing environment. In *Ayurveda* there are many texts which belongs to the ancient era denoting that diseases caught to human beings with their evolution. There is always involvement of underlying pathology which leads to manifestation of diseases. The pathogens grows within the body when they find suitable environment for growth. The diseases can be only controlled, when the growth of such pathogens last. There are many methods nowadays to inactivate multiplication of such micro-

organism/pathogens, one among them is “sterilization”.

Shalya Tantra is the one of the oldest text in *Ayurveda* written by *Acharya Sushruta*. The time period of *Acharya Sushruta* is said to be around 8th century B.C. The *Acharya Sushruta* is also known as “father of plastic surgery” even modern science accepted this thing, as it is the only oldest reference text available, which contain description regarding Rhinoplasty (*Nasasandhana*), Lobuloplasty (*Karnasandhana*) & Labioplasty (*Ostha sandhana*). This thing is very well known to us that procedures like this are not possible without proper sterilization of the articles and the equipment’s to be used for surgery. The *Sushruta Samhita* although is not a complete text available but there are many examples which shows importance of

Nirjivanukaran. The most common method used for sterilization/ *Nirjivanukaran* at that time was “*Dhoopana Karma*” which is also known as fumigation method. This method can be correlated with the O.T. fumigation method now days.

Sushruta Samhita

In *Sushruta Samhita* there is descriptions regarding the surgical problems & surgical emergencies which afflict mankind. Of all surgical emergencies, wound care is a major challenge to the surgeons, which has been described so extensively in *Ayurveda Shalya Chikitsa*. There are many descriptions in the ancient text which indicates that *Acharyas* kept utmost care of the wound /*Vrana*, either it is due to internal cause /*Nijvrana* or external cause/ *Agantuja Vrana*.

Acharya sushruta in *Agropharniya Adhyaya* described *Trividha Karma*. In which *Acharya* elaborately described the things required before surgical procedure (*Poorva karma*), at the time of procedure (*Pradhan karma*) and at the end of the procedure (*Paschata karma*). The articles that should be collected before the procedure includes *Yantra, Shastra, Agni, Shalaka, Pichu, Protta, Sutra, Patta, Kashaya, Lepa, Ushna Udaka etc* [1]

The role of “*Agni*” is to sterilize *Yantra, Shastra* and *Shalakas* (*Shalaka* is a type of *Yantra*) by directly heating on flame. Which is known as flaming method, it is a type of dry heat sterilization. “*Ushna Udaka*” is boiled water, which can be used to boil *Yantra, Shastra, Sutra & Pichu* in *Kattaha* (*Loha Patra*) to make it sterile. The boiling water above 100°C kills almost all micro-organisms. This method is known as boiling method & it is again a type of heating method. The use of *Kashaya* is for *Vrana Prakshalna* with *Jantughna Dravyas* that kills or remove micro-organisms from the operative site, nowadays we are using betadine, spirit, hydrogen peroxide, boric acid, potassium permanganate for this purpose. *Acharyas* mentioned these drugs as “*Shodhna Kashaya*”

In *Shodhana Kashaya Shankhini, Ankotha, Maalti, Kaner, Suryamukhi* And *Aragvadhaidigana* drugs are the main drugs.^[2]

The “*Protta*” (piece of cloth) & “*Patta*”(bandage) can be sterilized by *Dhoopna* with *Rakshoghna Dravyas* with the powdered drugs like *Guggulu* (*Comiphora mukkul*), *Aguru* (*Aquilaria agollocha*), *Sarjarasa* (*Resin of shoeria robusta gaerten*) *Vacha* (*Acorus calamus*)

Sarshap (brassica nigra), *Lavana* (Sodium chloride), *Nimba* (Azardhiracta Indica mixed with /*Ghrita* of cow and is used for fumigation.^[3]

We can protect the wounded man from *Nischacharas* / micro-organisms by following the *Rakshavidhan*, & the *Yama*, *Niyam*.^[4] *Rakshavidhan* is one among *Shastiupkramas* (sixty measures for the management of *Vrana*) described by *Acharya Sushruta*.

Yama- Ahinsa, Satya, Brahmacharaya, Vyavhara, Nivratti are five *lakshnaas* of *Yama*.

Niyama- Akrodha, Guru Sushruta, Shocha, Laghu Aahara, Aapramaad are five *lakshnaas* of *Niyama*.

In *Sushruta Samhita* various surgeries like Ophthalmologic (Cataract surgeries), Abdominal, Urological, Anorectal, Plastic surgeries, Orthopaedic, Gynaecological and obstetrics etc are mentioned. *Acharya Sushruta* has described 101 types of *Yantras* known as blunt instruments and 20 types of *Shastras* known as sharp instruments for these surgeries. The *Acharya Sushruta* and *Vaghbata* has given classification of surgical instruments which holds good even today. The surgical instruments which we

are using today are modification of ancient surgical instruments, for the success of surgery they explained various methods of sterilization.

In *Shastravcharanvidhahyya*, heating up of instrument on open flame till the instrument become red hot and then immediately dipping it into liquid of *Kshar* (alkalies), *Udaka* (water) or *Taila* for few seconds is called *Paayana*/Tempering. The tempering keeps the instrument rust & infection free and the sharpness of blade become augmented and more effective. Rust makes the instrument non sterile and leads to severe infection like tetanus, hence tetanus prophylaxis is important before commencement of any surgical procedure, this example again proves ancient technique of sterilization.^[5]

Hands should be well lubricated with *Sneha* & the excessive nail growth must be excised so that it may not spread any infection or may not injure the site of surgical procedure.^[6]

The intelligent *Vaidyaa* should get this instrument prepared with pure & strong metal by clever & expert blacksmith.^[7]

Acharya Sushruta in *Dravdravya Vidhi Adhyaya* had written water purification

method for drinking purpose by using *Utpala, Naga, Champaka, Patala* and three stones, *Gomedha* (garnet), *Moti* (pearl) and *Sphatik* (alum).

Charka samhita

Sootikagaara- Acharya Charka has given beautiful description of “*Sootikagaara*”, In which he has mentioned that the site of “*Gaara*” should be free from all amenities that could be a source of infection like bone, sand, broken pieces of mud vessels. The water & air of that site should be best in quality. It indicates that Acharya’s know very well that infection can spread through land, air and water easily. The main door of the *Gaara* should face towards the east or north direction, So that the room may get proper sunlight and fresh air, as sunlight possess anti-microbial properties.

Sunlight inactivates microorganisms like bacteria, fungi, viruses and protozoa through a combination of DNA damaging effects of ultraviolet radiations and thermal inactivation from solar heating.^[8] Antimicrobial activity of simulated solar disinfection against bacterial, fungal and protozoal pathogens and its enhancement by riboflavin.^[9]

Bilva, Tenduka, Induka, Bhalatka, Varuna & Khadira Kastha should be used for preparation of *Sootika Gaara*, as these

drugs are antimicrobial in action. The fire, water, toilet & kitchen should be at suitable place so that the infection may not spread to the new born and the pregnant women.^[10]

Shishu Upchara- Updhana (gloves) must be worn after excision of nail for cleansing of *Talu, Ostha, Kantha, Jivha*, with Cotton swab.^[11]

Nadi kalpna - (Naalchedhna) Excision of umbilical cord should be done with half edged sharp instrument made up of silver, gold or iron (silver and gold possess anti-microbial property) at 08 A distance from its attachment from abdomen. In case of occurrence of any infection in the umbilical cord after excision, oil prepared from *Kalka* of drugs like *Lodhra, Mulethi, Priyangu, Devdaru, Haridra* should be applied along with *Churna* of these drugs.^[12] These drugs are anti-microbial & ant-inflammatory in properties. Acharya’s kept this thing into mind that all the *Upkarnas* used at the time of *Naalchedhna* must be properly sterile so that it may not lead to any type of infection.

Measures for protection of mother and child- After *Jatakarma* proper measures should be taken for the care of mother and child. Drugs like *Aadani, Khadira, Karkandu, Peelu, Parushaka*, should be tied all around *Sootikagaara*. Drugs like *Vacha,*

Kutha, Gathivan, Hingu, Sarshapa, Tesi, Lahsuna, Kankarnika, Guggulu should be tied on the main entrance in the form of *Potli*. Same drugs should be used to tie around the neck of the mother and child. These drugs should be tied around the each and every utensils or items used for mother and child like water pot, bed & doors. In *Sootikagaara, Kankanika* and *Tinduka* should be used as a fuel for burning continuous fire.^[13]

Dhatri karma- "Shudha Dugda" means the mother milk must be free from *Asthakshira doshas* in other words we can say that it must be sterile, "*Snatanulipta*" means coating of the drug like *Chandana* is done around the breast. "*Shukla Vastra*" means the *Dhatri* should bear cloths that are not contaminated and *Aushdha Dharna* with drugs like *Aendri, Brahmhi, Shatvirya, Sahasravirya (Shweta Durva), Patla, Guduchi, Hare, Aaristha (Nagbala), Vatyapushpi (Mahabala), Vishvakasenkanta (Varahi Kanda)*, so that any type of infection may not caught to *Dhatri* or new born from any source.^[14]

Kumaragaara- It should have proper sunlight, devoid of free flowing air but ventilated, well protected from entry of ferocious animals, rodents, biting animals

and insects. Suitable place for fire, bath, toilet & kitchen should be provided in the *Kumaargaara*. "*Suvihitrakshavidhana*" means in *Kumaargaara* complete safety measures must be adopted.^[15]

We can reuse cloths, bedding & bed sheets of new born after sterilization by *Dhupana Karma* (Fumigation). The *Dhupana Dravyas* like *Yava, Sarshapa, Atsi, Hingu, Guggulu, Vacha, Chorpushpi, Brahmi, Sahasravirya, Jatamamsi, Palanksha (laakha), Ashoka, Kutki, Sarpa Kenchul* (skin of snake) should be chosen for *Dhoopana Karma* of cloths, bed sheets, and top sheets because these drugs possess antimicrobial property.^[16]

Mani & Aushadha Dharana- *Mani* & the drugs like *Aendri, Jivaka* and *Rishbhaka* should be used for *Dharana*. It protects from infections that could spread post delivery to the new born. Rhinosarus, *Mriga*, blue bull, and rt. horn of bull must be tied to the neck or arm of the new born.^[17]

Ashtanga Hridayam

Acharya has mentioned *Prakshalan* and other *Upkramas* for treating infected wounds with *Aragawadi Gana* and *Sursadi Gana* similarly they told to use *Kashaya*

drugs, like *Aguru, Sarshap, Hingu, Raala,* Salt and *Neem* leaves. ^[18]

In postoperative condition *Acharyas* mentioned *Prakshalan* with *Kashaya* drugs, *Dhupana* with *Rakshoghna* drugs, then covering the wound with *Kalka* of these drugs & finally covering the entire wound with *Kavlika*.

Bandaging should be done with *Patta, Kavlika & Vikashika*, made up of clean, soft & thin cloth after fumigation. It should be kept in sunlight to clear moisture. ^[19]

Hansodaka- As we all know that water is essential for human survival and it is very important that the source of water should be clean and free from any type of contamination. In *Ayurvedic* texts many different sources of water has been explained like *Antriksha Jala/Indra Jala/Gangambu* , *Jala* from rivers (himalaya rivers & *Malya* rivers), *Koopa Jala* all are *Pathya*. The *Acharya Vagbhata* & *Charka* also described filtration method in his text and named as "*Hansodaka*". The *Hansodaka* is the water collection in the metal vessel under direct sunlight and then cooled under moonlight in the *Agastya nakshatra*.^[20]

As the *Hansa* drinks only pure water, thus *Acharyas* named it as *Hansodaka*. The name indicates the purity of water collected at that time. The "*Agastya*" is the star named after *Agastya Rishi*. The "*Aga*" means mountain and the "*Asti*" means thrower. The *Agastya* sets around march-april and further rises in the south in sept. The color of *Agastya* star changes to different colors appearing like conch shell, jasmine, cow milk, lotus roots or silver shades which is the sign of benevolence, while appearing like a blaze flesh or blood, leads to war, riots and calamities.

During rainy season particularly when sun transits in the tropical *Karka & Simha*, all rivers become untouchable & considered to be going under menstrual cycle means, rivers are not pure. With the rising of *Agastya*, river and ground water starts their purification naturally, this is the suitable time for making dips as the river water is purified and that water can also be used for drinking purpose.^[21] This is the only reason that why *Acharya's* mentioned *Agastya Nakshatra* for *Hansodaka*.

Kashyapa Samhita

In *Kashyap Samhita* in *Dhoopna Dravya Adhyaya* various *Dhoopana Dravya*

combinations are mentioned for all diseases like *Dhoopna* with *Ghrita*, *Padmaka*, *Khas*, *Netrabala*, *Nagkesar*, *Rasa (Sarja Rasa)* [22] *Dhoopna* with *Ghrita*, *Neema*, *Tulsi*, *Kaner patra*, Hairs of cow, sheep & goat for all diseases. [23] Drugs like *Karanja*, *Kapas*, *Vacha*, *Sarshapa*, *Bast loma*, *Hingu* can be used for *Dhoopna Karma*. [24] *Rakshoghana dhoopa- Ghrita*, *Shweta Sarshapa*, *Hingu*, *Devnirmalya*, *Akshat (rice)*, *Skin of snake*, *Bhiksu Sanghati* (old cloths of *Bhikshu*) [25]

In *Dvivraniya Chikitsa Adhyaya Kalka* of drugs like *Haridra*, *Daruharidra*, *Tila*, *Ghrita*, *Saindhava*, *Mulethi*, *Trivrta*, leaves of *Nimba* considered as *Shodhana* and *Ropana* in property. [26] Bandages are important to protect wound from air, sunlight, foreign body like *Kaastha*, water and *Makshika* (house hold fly). All these sources may contaminate wound and deteriorate the condition of the patient hence *Acharya's* used bandages after proper fumigation with *Dhoopna Dravyas*. In modern sterilizing techniques we are using autoclaves for sterilization of bandages, cotton, gauze piece etc. [27] Sterilization is necessary for the complete destruction or removal of the micro-organism Modern techniques are broadly classified into physical & chemical methods.

PHYSICAL METHODS-

1. Heating method a) Dry heat b) Moist heat
2. Radiations
3. Filtration
4. Sonic ultrasonic vibrations

Dry heat-The Sterilization with heat but without water or very little of it by conduction, the dry heat destroys microorganisms by coagulation of proteins.

Dry heat methods-

- Bunsen Burner
- Hot air oven
- Infrared radiation
- Microwave
- Electric cautery
- Flaming
- Incineration

Bunsen burner- It is equipment that most commonly used in laboratories to produce a single open flame. It is used for Sterilization by heating.

Hot air oven (poupinel) – Electrical device used in Sterilization with digital control to maintain temperature. It is a double walled insulated device, the insulation keeps the heat & conserves the energy. There is also air filled space in between insulation for uniform distribution of heat. It is fitted with

adjustable aluminum trays. It is used to sterilize glassware, powder, fat & oil with 0.5mm thickness for 1hr at 190°C temperature for 6-12 min or 160°C ≥ 2h.

Disadvantage-

- ✓ Organisms like prions are not killed.

Infrared radiation (IR) - Infrared radiations are part of electromagnetic spectrum, with in the spectrum infrared waves occurs at frequencies above those of microwaves & just below those of red visible light, hence called as “infrared”. These radiation ranges from 700nm to 1mm and travel from the source with speed of light passing through air without heating directly targets object. All objects in the universe emit infrared rays but main sources are sun and fire. IR Invisible to human eye but can be felt as heat. It is of different types depending upon range of wavelength. Near, short, mid, long & far infrared. In this method exposure to infrared rays for 7.5 min. at temp. upto 180°C is given by placing substances on moving conveyer belt and passing through a tunnel which is heated by using infrared radiations. This method of sterilization is suitable for heat resistant instruments like syringes, glassware & metallic instruments.

Microwave- The kitchens are a commonest source of contamination, as microorganisms gain access from uncooked food items to utensils, sponges and dish cloths. The microwave ovens are so quick and efficient because they conduct heat energy directly to the molecules inside the food.

Electric cautery- It is an electric device in which direct AC is used to generate heat energy between 100°C to 1200°C by passing through a resistant metal wire electrode. The heated electrode is applied to the living tissue at different temperature to generate intracellular heat, which can cause cutting (boiling + explosion), desiccation (dehydration), Fulguration (fire sparks) and coagulation and the process is known as Electro cauterization. The process can be performed by using either unipolar /monopolar or bipolar electric cautery.

Flaming -This method is commonly used for small metal or glass objects. Flaming is to dip the object in 70% ethanol. The ethanol will ignite & burn off rapidly leaving less residue than a gas flame.

Incineration- This Method is used to burn organic substances contained in waste materials.

In case of dry heat sterilization the chemical indicator tapes having Signals in V shape

are used for confirmation, the tape converts themselves from light green into intense green.

Biological indicators are also used.

Disadvantage of dry heat-

- ✓ This method requires longer exposure.
- ✓ Time consuming process.
- ✓ Require higher temperature than moist heat.

Moist Heat

At temperature below 100° c

- ✓ Vaccine bath -Vaccine sterilization
- ✓ Serum bath
- ✓ Inspissation
- ✓ Pasteurization- Food industry

At temperature 100° c

- ✓ **Boiling method-** In this method instruments are boiled with water & 2% sodium bicarbonate added to it, and boiled for 30-45 minutes. This method is suitable for Instruments (blunt), rubber material, catheters drainage tubes & the gloves. Sodium carbonate does not allow instruments to catch rust.

At temperature above 100° c

Autoclave- An autoclave works like a pressure cooker. It transfers heat energy to an object to be sterilized. It is suitable for objects that can tolerate humidity, high

temperature (121° c) and pressure (15 lbs.) i.e. stainless steel instruments. It is filled with water and the material to be sterilized are kept into it in a stainless steel drum & then properly sealed. Now high temperature steam is generated through the electric immersion rod, which is connected to source of current and steam is forced under high pressure, thereby displacing all initially present air in an autoclave. The autoclave is fitted with a safety valve to prevent explosion if the steam pressure gets too high. The moist heat kills all microorganisms by irreversible coagulation and denaturation of enzymes & structural protein. This method is very effective against spore forming bacteria & viruses it is important that the steam must be in direct contact with the material to be sterilized. The composition of steam with in an autoclave is 97% gas and 3% liquid, with change in percentage sterilization time changes.

To ensure proper sterilization Chemical indicators & biological indicators are used. The *Chemical indicator tape* is placed on packages containing articles prior to sterilization. These tapes are self-adhesive, lead free heat resistant & moisture resistant, made up of semi-creped paper. It easily sticks on plastic, metal items, fabrics,

cardboard and paper etc. The indicator changes its color when exposed to steam, providing a visual confirmation. i.e yellow printed strips turn to brown after sterilization.

The **biological indicators** contain spores of the heat resistant microbes i.e. *Bacillus atrophaeus* (BA), *Geobacillus stearothermophilus* (GS) to determine that autoclaving cycle were adequate to inactivate microbes. These indicators are placed in locations where it is difficult for steam to reach to verify that steam is penetrating there. It can be used in the form of spore containing glass vials, liquid media or strips.^[28]

RADIATIONS- Radiation is the emission or transmission of energy in the form of waves or particles through a material medium. It is of 2 types

- 1) Non- ionizing
- 2) Ionizing

NON- IONIZNG - These are the radiations of longer wavelength with low intensity. It is used only for surface sterilization.

- U.V. Radiations - The sun emits three types of UV radiation UV-A, UV-B & UV-C. The UV-C (100-280nm) radiation is absorbed by the outer dead

layer of human skin, while UV-B (280-315nm) & UV-A (315-400nm) radiation penetrate deeper.^[29] U.V. radiations are not considered as good sterilant, as it is unable to kill all microorganisms ,but it is a good disinfectant. There are certain studies that shows that UVGI (ultra violet germicidal irradiation) can be safe and highly effective in disinfecting the water, surfaces & air, which can prevent transmission of airborne infections. Low pressure mercury lamps are used in UVGI applications which emit short wave ultraviolet-c, 100-280 nm, it kills or inactivates microbes by deactivating DNA.

- MICROWAVES (MW) - It is a thermal process that delivers energy to the food packages under controlled temperature to inactivate harmful bacteria's. Microwaves interact with polar water molecules and charged ions, the friction resulting from molecule aligning in rapidly alternating electromagnetic field generates the heat with in the food. Since heat is directly generated in the food, the thermal processing time is reduced to great extend.

- INFRARED (IR)- Discussed earlier in dry heat methods.

IONIZING- These are the radiations of shorter wavelength with higher intensity. Ionizing radiation inactivates microorganisms by reacting with DNA and key enzymes. It produces disruptions in subatomic particles involved in the formation of microorganism.

- Gamma irradiation
- Electron irradiation
- X-ray irradiation
- Gamma rays- gamma radiation emitted from radioactive material like isotope cobalt- 60 or cesium- 137 has more penetrating power, it is used to sterilize medical equipment, like needles, syringes, cannulas, IV sets, food etc. It is also used to kill cancer causing cells.
- Beta particles-beta particles are more ionizing than gamma rays and will cause more damage for the same exposure and intensity. Beta particles passes through skin and can be used in medicine. Mostly energy of 10 MeV is used for sterilization.
- X-rays- It is of short wavelength with more penetrating power than gamma rays and electron beam, can easily pass through the body to treat internal

organs. Energy of 5-7 MeV is used for sterilization.

Disadvantages-

- ✓ It is expensive.
- ✓ It may damage some of material to be sterilized.
- ✓ It is dangerous, if not used properly.

FILTRATION- It is the method used to sterilize solutions by passing through filters capable of removing microorganisms. The size of filters must be less than 0.75m, which can retain tiny microorganisms. As the pores are too small it requires high pressure or suction to carry out the process of filtration.

TYPES OF FILTERS-

- 1) Chamberland filter
- 2) Berkefeld filter
- 3) Seitz filter
- 4) Sintered glass filter
- 5) Cellulose membrane filter

Chamberland filter- This type of filter is made up of unglazed porcelain candles, with minute pores of various size measuring 0.1-1micron (100-1000nm). It removes all micro-organisms & makes the liquid sterilize. Depending upon size of pores it is of different type that is L₁, L_{1a}, L₂, L₃, L₅,

L₇, L₁₁, and L₁₃. L₁ is the coarser and L₁₃ is the finest one.

Disadvantage- It can't check viruses.

Berkefeld filter- It is a water filter made up of diatomaceous earth also called diatomite or Kieselguhr. It is a fossil remains of diatoms, a type of hard shelled algae. Diatoms are single cell plants of microscopic size. The size of its particle vary from 10 to 200 µm. It occurs naturally as a soft siliceous rock, which is very light in weight due its high porosity.

The property of porosity makes it useful for water filtration, that removes microorganisms like bacteria. It removes solid particles, Cysts, pathogens like E-coli, Shigella, Klebsiella, and also prevents diseases like Typhoid & Cholera. The candles is impregnated with silver to inhibits microbial growth, giving the candles self sterilizing properties, such filters works on gravity and the method is also known as gravity filtration.

TYPES-

- 1) Viel- Coarsest pores
- 2) Normal- Intermediate pores
- 3) Wenig- Finest pores.

Disadvantage-

- ✓ Doesn't check viruses.

CHEMICAL METHODS-

Ethylene oxide (C₂H₄O) - Ethylene oxide is a highly inflammable gas which is colorless, sweet in odor like ether & having boiling point of 10.7°C at 760 mm Hg. The gas is mixed with carbon dioxide or nitrogen to make it stable and used to sterilize heat sensitive medical equipment's which can't be sterilized by autoclave. The EO sterilization method works under five different stages that is 1) Evacuation with humidification 2) Gas introduction 3) Exposure 4) Evacuation 5) Air washes. EO must have direct exposure to microorganisms or items to be sterilized because it is a surface disinfectant. EO chemically reacts with protein & DNA structure of microbes and prevents its reproduction. The indicator tape changes its color when exposed to EO, the brown printed strips turn to green after sterilization.^[30]

Disadvantage -

- ✓ Highly inflammable.
- ✓ Toxic in nature.
- ✓ Time consuming process.

Peracetic acid (CH₃CO₃H) - The Peracetic acid (PAA) is an organic compound made

up of equilibrium mixture of acetic acid and hydrogen peroxide and water. The liquid is colorless & clear in appearance with pungent smell. The PAA gets decomposed under high temperature hence should be kept under cool atmosphere. It hydrolyses and decomposes rapidly when diluted with tap water. The content, Acetic acid and hydrogen peroxide is responsible for this hydrolysis. On its decomposition oxygen gas is liberated. The PAA penetrates through the cell membrane of microorganisms like bacteria, yeast, molds and viruses and irreversibly interrupt the enzyme activity and lead to destruction of the microorganisms. Hence it is a good oxidizing agent and disinfectant. It is useful for unsaturated fats, synthetic and natural rubbers, also useful for heat sensitive medical equipment's like endoscope.^[31]

Disadvantage-

- ✓ Skin, eyes, nose and throat irritation.
- ✓ Causes Asthma.
- ✓ Prolonged exposure can cause lung damage.

Formaldehyde gas- It is used as fumigant in gaseous form for sterilization of operation theatres.

Disadvantage-

- ✓ Less efficient method.
- ✓ Time taking process.

Glutaraldehyde (C₅H₈O₂) – The chemical glutaraldehyde is a colorless liquid with pungent odor. It is a cold sterilant that kills microorganisms including viruses and spores. It is used in 0.1 to 1% concentration to disinfect heat sensitive instruments, like endoscopes, bronchoscope and dialysis equipment's. It can be used either by soaking instruments or by performing maintenance procedures by automated processors (i.e. Filter and hose changes), there are several other methods by which it can be used. The use of automated processing equipment to disinfect instruments reduces glutaraldehyde exposure remarkably. Over exposure to glutaraldehyde vapors may prove harmful to workers, it should be handled in a controlled manner and proper precautions must be taken. In spite of this fact it is a safer alternative to formaldehydes. Adding glycine can safely deactivate glutaraldehyde. Useful in heat sensitive instruments.^[32]

Disadvantage-

- ✓ Dermatitis, Asthma

Chlorine dioxide Gas (ClO₂) –

Chlorine dioxide gas is used to sterilize medical and laboratory equipment, surface, rooms and tools. Chlorine dioxide can be used as oxidizer or disinfectant. It is a very strong oxidizer, that kills pathogenic microbes like bacteria, fungi & viruses effectively. It is used in liquid form to sterilize medical and laboratory equipment, surfaces, rooms and tools.^[33]

Disadvantage

- ✓ Chlorine dioxide gas produces mucosal eye and respiratory irritation at concentration of 5ppm.
- ✓ Should be under taken after careful safety analysis.

Hydrogen peroxide (H₂O₂)-

Hydrogen peroxide is a clear, colorless liquid chemical compound which is slighter viscous than water & bitter in taste. Hydrogen peroxide is formed by Anthraquinone process. Hydrogen peroxide is a unstable compound which decomposes slowly in the presence of light, hence it is stored with a stabilizer in a weak acidic solution. It should be stored in a cool and dry place away from inflammable substances, in a nonreactive materials such as stainless steel, aluminum, plastic, or glass. It is used as an oxidizer, mild

antiseptic & bleaching agent .When it is applied on the affected site, it releases oxygen with foam formation which is known as oxidation. This foam removes dirt, debris & dead tissues out of the affected area. Hydrogen peroxide is also formed with in the body by immune system in the controlled manner, the phagocytes use this hydrogen peroxide to kill engulfed pathogens.

Hydrogen peroxide can be used in different concentration ranging from 2% to 40%. In low concentration 3-9 % it can be used for medical purposes. In High concentration used in textiles & paper industries, above 40% it is hazardous. It is an effective compound against viruses, yeast, fungi, bacteria & its spores. It acts as sporicidal when used for longer period. It is useful in the sterilization of heat or temperature sensitive equipment's like endoscopes. It is alternative to chlorine based bleaches.^[34]

Disadvantage-

- ✓ Redness & irritation at site of application.
- ✓ Irritation to the eyes.
- ✓ Rashes on application area.
- ✓ Vapors in high concentration may cause headache, dizziness, vomiting & diarrhea.
- ✓ Risk of cancer.

Ozone (O₃)-

Ozone which is also known as "Trioxxygen" is the natural gas, having pale blue color, pungent smell & strong oxidizing property. The ozone has ability to kill bacteria, fungi & viruses and parasites that causes air contamination. It is used at low concentration (5 mg/l) for 15 min, in vapor form to sterilize laboratory tools, gloves, small instruments, endoscopes. It is capable to kill 99.7% of 650 different kinds of pathogenic organisms in 90 min. It is more penetrating than hydrogen peroxide but lesser than ethylene oxide. It is the best technology available and is gradually replacing older methods of sterilization. It is useful in sterilization of lab tools like pipettes, pipette tips, gloves, plates small instruments.^[35]

Disadvantage-

- ✓ Highly toxic.
- ✓ Unstable gas.

Nitrogen dioxide- It is a sterilant gas that is used in the terminal sterilization of medical instruments. The sterilization is done by chamber based system. It another type of low concentration sterilization, rapid in microbicidal activity. The boiling point of the NO₂ is 21°C at sea level, which is

relatively high saturated vapor pressure at ambient temperature, because of this, the concentration of NO₂ used during a sterilization cycle is far below the NO₂ dew point, and condensation of the sterilant will not occur. Also this gaseous nature of NO₂ at ambient condition allows for efficient aeration of the load, making the processed article safe to handle. Useful in sterilization of medical devices packed in a porous packing.^[36]

NEWER METHODS OF O.T. STERILIZATION

The old method of sterilization by formalin is more prevalent in many countries although because of its toxic nature it is no longer in practice in many developed countries. The agents like hydrogen peroxide (for gas plasma sterilization), hydrogen peroxide with silver nitrate, peracetic acid (peroxyacetic acid) and chemical compounds other than formaldehyde like paraformaldehyde are safer and should be used.

Bacillocidrasant: It is a newly prepared compound which is more effective and less expensive. It provides germ free environment within a short period of 30 to 60 minutes, thus shutdown of operation theaters for longer duration unlikely to old

formalin fumigation method doesn't required.

Virkon: A non- aldehyde chemical compound which is proven to have wide spectrum anti- microbial activity, moreover it is a non- toxic compound hence better than formalin which is a potent carcinogen. Virkon is composed of oxone (potassium peroxy mono sulphate), sodium dodecyl benzene sulfonate, sulphamic acid & inorganic buffers. [37]

CONCLUSION

There are different techniques in modern science for disinfection and sterilization, but it seems to be work on the same principles as mentioned by our *Acharyas*. It has been concluded that in modern method of sterilization we are using chemicals and

gases which causes serious adverse effects to the health of human beings. In such case *Ayurvedic* methods can become an alternate if used in combination with modern methods. However research is required in this field to develop newer techniques which will be more safe to human & environmental health.

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