CHAPTER IV

ANTIMICROBIAL ACTIVITY
CHAPTER-IV  
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IV.1 INTRODUCTION:

We know that the necessary requirement of all living things is water. There is no life without water. This is a common saying depending upon the fact that it is a master solvent. All metabolic reactions of living beings depend on the presence of the water. All human population living in towns, cities, villages, etc. is dependent upon municipal or grampanchayat supplies of water. Besides, a major fraction of our population which lives in rural areas, particularly in underdeveloped and developing countries, is dependent upon lakes, rivers, wells, ponds, springs, etc. for their water requirements and needs.

The water is lost from the earth by different ways such as evaporation, transpiration and exhalation and comes back to the earth by the way of precipitation. The contamination of water starts right from the beginning when water reaches the earth through air in the form of precipitation. The microorganisms present in air get entry into it. After the precipitation is over and water reaches the earth surface, it is contaminated by microorganisms via soil, soil deposits, dead plants and animals, etc. The natural water supply sources are getting contaminated by a large array of substances such as domestic and industrial wastes, i.e. sewage, discharged as a consequence of civilized man’s need, and human and animal excrete in the form of urine and feces. All these contaminants deteriorate the quality of water and make it unsafe for the human consumption [Jamdar et al., 2013].

The contaminations, particularly domestic and industrial wastes and fecal ones are of much biochemical concern disease causing microorganisms. These disease-causing microorganisms occur in the feces and urine of an infected person. After the discharge, they are mixed in the drinking water supply source. This generally results in transmission of disease from
the infected to healthy persons. The diseases transmitted via water are always called as water borne diseases.

It is well known fact that the origin of the matter is in the water and matter is sustained in the water. All the living things depend only on the water. We use water for different purposes viz. agriculture, industry, drinking and disposal of waste. It has been considered that the earth formed constitute ocean and atmosphere. In the atmosphere hydrogen and oxygen combines to form water.

**IMPORTANCE**

Water is not only essential to life but is the predominant inorganic constituent of living matter. It makes up some 5 per cent of the body weight of an adult human and can form as much as 98 per cent of the mass of certain jellyfish. Organisms which contain relatively small amounts of water are generally in dormant state or show very slow development; seeds and certain invertebrates that live in arid environments are examples. On the other hand, high rain fall over a land mass invariably means a large biomass per unit area.

**MEDIUM FOR GROWTH**

Microorganisms are mostly grown in the soil and water. The different constituents, compositions and contents are related to the growth of microorganisms. The mineral nutrients and oxygen present in the dissolved form in the water is responsible for the growth in the water. There is direct proportionality relation between the bacteria and the organic matter. If the amount of organic matter increases, then the growth of bacteria or the microorganisms goes on increasing up to the certain limit.

The number of bacteria and other microbes will always be higher in river passing by thickly populated cities then of the villages because persons living in cities, are continuously disposing sewage water and other waste products in rivers which contain a very amount of mineral nutrient a medium for their growth. Moreover, the pH, temperature range and inorganic phosphate content as well as the situation of the lake and river also support the growth and cause a dense population of microorganisms. These organisms (bacteria, blue green algae, etc.) form heavy blooms under these conditions. The possible factors responsible for limiting the growth and density of microorganisms are the available amounts of zinc, copper, and the poor quantity
of nitrate nitrogen etc. It has been noticed that the excess of calcium is harmful for the luxuriant
growth of microorganisms, especially to algae in general. However, Insipite of enormous quantity
of the substance that exists, only a small proportion of it is actually usable by human beings.
Humans use water in the home, in industry, in agriculture, and for recreation.

In one way or the other we use all available sources-inland water, and even ocean water. We pollute it re-purify it, and reuse it over and over again. Of the three states in which water
occurs in the ecosphere, gas, solid and liquid-only the last is an indispensable resource.

The different forms of water are found in every section of ecosphere; the atmosphere, the
lithosphere and the hydrosphere (a term designating the entire system of seas and inland waters).

All this facts and factors pointed to the necessity for employing water treatment
technique which can provide safe potable or non-polluted drinking water i.e. water free of
pathogenic microorganisms and harmful chemicals.

**IV.2 ORIGIN OF BACTERIA**

Waters can be divided into four well marked classes as atmospheric water, surface water,
ground water and stored water. They are as follows:

**IV.2.1 ATMOSPHERIC WATER**

Rain-water and water formed by snow are grouped under atmospheric waters. As the rain
and the snowfall, they wash the dusty-atmosphere and bring down dust, soot and other suspended
particles to the earth. Along with these non-living particles atmospheric water also carries air-
borne bacteria to the earth’s surface. In rain water number of bacteria may be up to a few
hundred per ml.

**IV.2.2 SURFACE WATER**

As soon as rain drops and the snowflakes reach to surface of the earth, they quickly
become contaminated with the soil micro-flora of the area. Such waters are then referred as
surface water. Mountain water contains a few microorganisms but as it flows from higher
regions to lower regions, it contains large amounts of organic matter, the population of these organisms’ increases considerably. Such water facilitates growth of the saprophytic species and protozoa. River water often shows their highest count during rainy season.

IV.2.3 GROUND WATER

As water percolates through the earth, it is usually filtered, that is why it reaches to certain depths on account of which it is named as grouped water. Such water supports the growth of a few types of the bacteria. They may be 10 bacteria per ml. The ground water contains a little amount of organic matter. This is the reason that the microorganisms cannot multiply suitably in such water. Such organic material can prevent the growth of bacteria in such water environment. Other organisms may be prevented to grow.

IV.2.4 STORED WATER

Water which is present in ponds, reservoirs, lakes or ocean for a considerable period is called stored water. The microbe population decreases greatly in such waters. The number of these organisms depends of the environmental conditions of the area, and the inorganic solvents present. Protozoa which are abundantly present in stored water play a significant role in decreasing bacterial population by using them as a food. Protozoa require living and dead bacteria for their food.

Protozoa, in stored water, can only survive in presence of bacteria and dissolved oxygen. The appearance and growth of certain sheathed bacteria and algae in such water, after their death and decomposition, create a disagreeable odor and taste. This activity of microorganisms poses a serious problem in water supply.

IV.2.5 MICROBIOLOGY OF LAKE

The content of organic material is responsible for the growth of organisms in the stored water in the lakes. When the lake is situated near the roads having high traffic, then the lake water is contaminated by many waste materials. These further can cause in to organic material
formation. The lake water can be divided into three parts as entropic lake, oligotrophic lake, and dystrophic lake.

**IV.2.6 ENTROPIC LAKE:**

The Lake which is well nourished, receives water from a stream draining areas, and rich in plant-vegetation is referred as eutrophic lake. This kind of lake contains a high amount of organic matter and minerals which support the luxuriant growth of micro and macroscopic aquatic organisms.

**IV.2.7 OLIGOTROPHIC LAKE:**

Such types of lakes are poorly nourished and are found to be less productive. The organic and mineral contents of such lakes are very small and are not capable of supporting the growth in aquatic life. These lakes are considered to be having some safety or some safety precautions are taken nearby its surroundings.

**IV.2.8 DYSTROPHIC LAKE:**

These lakes contain high organic matter of special type, such as the remains of incomplete digestion of matter in the area draining into the lake. Dystrophic lakes are often dark in color, and acidic in nature. Due to the acidic nature, a very few species of bacteria and other forms of aquatic life are able to grow.

**IV.3 IMPURITIES IN WATER**

We know that, it is not possible to obtain 100 percent pure water either naturally or artificially. All are working with fresh water and it is used in the all types of research in the laboratory. But the main concern is always the purity of fresh water. The percentage of
distillation cannot be achieved or increased at very high level. Hence the microorganisms grow in any type of water. So the concept of microbial activity is concerned with human life or all the lives on the earth. It is general practice that one mg of impurity in the one liter of water can be represented by 1 ppm.

Since the water chemist is usually working with fresh water, the impurity level is measured in parts per million. Since a liter of distilled water weighs 100g or 1,000,000 mg, it is apparent that 1 mg of impurity in a liter represents 1 ppm.

However, a liter of sea-water weights about 1032g, so 1 mg of impurity in sea-water is less than 1 ppm. Because the density of the water may be quite high, the use of milligrams per liter is more precise than parts per million, although for practical purpose they are identical when dealing with fresh water.

### IV.4 WATER POLLUTION AND ROLE OF MICROBES

Modern civilization is dependent on water for irrigation, industry, domestic needs, shipping and of increasing importance for sanitation and disposal of waste. Most of our water bodies, such as ponds lakes, streams and river have become polluted as a consequence of increasing industrialization, urbanization and other development activities.

The study of microbes concern with rivers reveals that water in the river consists of some nutrients as well as highly toxic substances viz., Nitrates, selenium, cadmium, mercury, chlorinated hydrocarbons etc. In U.S.A every major river has become seriously polluted. The great lakes which furnish the greatest single source of water to America and Canada have already been polluted. Massive pollution turned Rhine into a dead river in the heart of Western Europe. These suggestive measures are very important in concern with other other countries and India. The microbes are transferred from one place to another even at very long distances also. The population of microbes increases very fastly at higher temperatures.
In our country also some rivers are polluted in some or greater extent. Many parts of the river do not seem to have any dissolved oxygen and no wonder that they fail to supports the growth of desirable aquatic fauna and flora. Only such organisms and bloodworms, sludge worms, bloodsuckers and certain undesirable animals, pathogenic bacteria and pests are found. River Mini-Mahi in Baroda is another heavy polluted river which is loaded with a variety of industrial and petrochemical wastes.

The river Coolum flowing through Madras is polluted by sewage so much that not even the zooplanktons are able to thrive in it. One liter of Coolum water contains as much as 900 mg of iron, 275 mg of lead, and 1313 mg of nickel and 32 mg of zinc. Beside heavy metals, very high levels of phosphates, silicates and nitrates were also found in the water. Sulfate levels ranging from 80-408 mg l-1 were the highest recorded among Indian rivers. Many sugar mills and distilleries can produce troubles of polluting a various rivers. Such has been the extent of pollution caused that thousands fish will die in the river which will provide an excess organic matter for microbial growth.

IV.5 WATER POLLUTION

Water is said to be polluted when it is changed in its quality or composition directly or indirectly as a result of waste disposal and other human activities. The polluted water consists of very harmful metal or alloy elements which are toxic to human life. The dissolved gases such as hydrogen sulfide or nitrogen, the dissolved impurities such as mud, silt etc., and minerals such as magnesium or calcium also comprise the pollution. The microorganisms developed or present in the water results in to the pollution. Once the water is polluted, it is considered to be turbid and not pleasant for the drinking or any other purpose. The polluted water causes various diseases such as typhoid, cholera etc.

SOURCES OF WATER POLLUTION
1. Sewage and other oxygen demanding wastes, which contain decomposing organic matter and pathogenic agents;
2. Industrial wastes which contain toxic agents ranging from metal salts to complex synthetic organic chemicals;
3. Agriculture waste, which comprises fertilizers, pesticides and biocides; and
4. Physical pollutants viz., heat (thermal pollution and radioactive substances.

IV.6 PHYSICAL CHARACTERISTICS OF WATER

These are color; odor, taste movement, temperature, transparency and turbidity are the physical factors of importance in water.

1. COLOR:

Color in water means those, hues inherent within the water itself which result from colloidal substances and materials in solution. In naturals waters color may occur due to the presence of humic acids, folic acids, metallic ions, suspended matter phytoplankton, weeks, and industrial effluents. Algal flora imparts green color to water while water with excess of silt appears brownish. Organic matter and iron impart a yellow hue to the water.

2. ODOR AND TASTE:

It is very popular that odor and taste are the prime considerations for potable water. Natural water depending upon their impurities has specific odor and taste. Peaty, silica and chalky waters have an earthy odor. Decaying water weeds like Chara-rotten hay and straw impart an odor like that of decaying fish. Contamination with sewage water may give the odor of hydrogen sulfide. A fungus growing on decaying plant material yields a musty odor. Chlorinated water with phenol traces gives very strong chloro-phenol odor. Taste of water
too depends upon the impurities in water and it is also linked with the odor. There is much difference in taste and odor.

3. TEMPERATURE:

The sun radiations produce atmospheric effects. When these radiations have high energy, the water naturally gets heated. Also the atmospheric changes cause to raise the temperature of water.

Discharge of heated effluents also brings about thermal changes in natural waters (thermal pollution). Temperature is basically an important factor for its effects on chemical, biological reactions and growth of microorganisms in water. This increased water can speed up the chemical reactions.

4. TRANSPARENCY (LIGHT PENETRATION):

It is shown that turbidity of the water is directly proportional to the transparency of the water. It also depends on the matter existing in either organic or inorganic form. Thus in order to obtain the transparent and clear water, the factors causing impurity must be studied.

5. TURBIDITY:

Turbidity in natural waters is caused by suspended matter like clay, silt, organic matter, phytoplankton, and other microscopic organisms. It is actually the expression of optical property (Tyndall effect) in which the light is scattered by the suspended particles present in water. Scattering of light is dependent upon the size, shape, and refractive index of such particles. Turbidity, when largely because of phytoplankton, is considered as an index of productivity, but on the contrary, when because of suspended matter other than phytoplankton it restricts the light penetration in water resulting in reduced primary production (photosynthesis). In rainy season,
the turbidity is generally large. The rain water mixed with soil, particularly with clay or silt can add turbidity.

**IV.7 BIOLOGICAL CHARACTERISTICS OF WATER**

**The oxygen and the sewage relation**

There exist many waste products produced by household causes, plants and animals as well as environmental effects. If there exists not controllable dumping of the waste products then the pollution of water will result permanently in the lakes or storages. Thus sewage is produced. All the waste products decrease the level of oxygen present in that environment. The sewage causes to increase aerobic bacteria. The contamination of the water is largely related to the sewage or waste products. The aquatic bodies have a self-regulating mechanism for recycling of these wastes quickly. Normally, if the quantity of sewage received by water body is small, it is decomposed by the activity of aerobic bacteria in a short time. But, if the contamination level is higher, the self-purifying activity of the water body is lost and water becomes unfit for human consumption and other domestic uses.

A major ingredient of most detergents is phosphate. The phosphate combines and inactivates ions such as calcium so that they cannot react with and disable the dirt dissolving molecules. When discharged into water, the phosphate supports luxuriant growth or blooms of algae. Extensive growth of algae often withdraws great quantities of oxygen from water to the detriment of other organisms, and produces a bad odor when decay. Some decomposing plants are known to produce strychnine-like toxins which kill animals including cattle.

Discharge of untreated or partially treated sewage in water bodies as common practice followed by means of the municipalities, is one of the most common primary sources of water pollution, especially near big cities. This discharge of sewage results into the following:

**IV.8 MICROORGANISMS OF WATER:**
A large number of microorganisms (e.g. protozoa, bacteria, fungi, algae, nematodes) both saprophytic and pathogenic are found in aquatic environments. The type of microbial population differ in different type of water habitats such as unpolluted, polluted, and marine waters as stated below [Singh, 2009].

**MICROORGANISMS IN UNPOLLUTED WATER:**

*Paramecium, Actinomycetes, Yeasts, Bacillus spores, Clostridium spores, Cellulose digesters*

**MICROORGANISMS IN POLLUTED WATER:**

*Clostridium sp., Coliform Bacteria, Escherichia coli, Desulfovibrio sp., faecal streptococci, Prozian cysts, Blue green algae, enteric viruses*

**MICROORGANISMS IN MARINE WATER:**

*Mold spores, Halophilic organisms, Psychrophilic organisms, Dinoflagellates, Pseudomonas sp., Forminiferans, Luminous microbes*

**IV.9 WATER BORN BACTERIA:**

The bacteria are microscopic organisms with simple and primitive forms of prokaryotic type. Danish Physician Christian Grams discovered the differential staining technique known as Gram staining. There are two types of techniques as

1. Gram positive staining technique and
2. Gram negative staining technique

If the bacteria retain the crystal violet by resisting de-colorization with acetone or alcohol and appear deep violet then they are called Gram positive bacteria. The gram negative bacteria
are those in which bacteria lose the crystal violet. Then they are counter stained by means of saffrarin. This will be observed to be red.

The identification of bacteria is very necessary for observing their characteristics. These two groups of bacteria are recently classified into four different categories as follows [Holt et al. 2000].

i. The World of Bacteria I, This group consists of Ordinary Gram negative bacteria.
ii. The World of Bacteria II, This consists of Ordinary Gram positive bacteria.
iii. The world of Bacteria III, This group belongs to Bacteria with unusual properties.
iv. The World of Bacteria IV, This consists of Gram positive filamentous bacteria of complex morphology.

These types of bacteria are *Salmonella enteriditis*, *Aneromonas hydrophilia*, *Chromobacterium violaceus*, *Mycobacterium tuberculosis*, *Vibro-Cholerae*, *Campylobacter*, *Escherichia coli*, *Shigella*.

**IV.9.1 STAPHYLOCOCCUS AUREUS: FAMILY: (MICROCOCCACEAE)**

**Genus: Staphylococcus**

The word staphylococcus is derived from the Greek language (Gr. Staphylo = bunch of grapes, Gr. Cocus = a grain of berry). The species name was derived from Latin language (L. *aureus* = golden). *Staphylococcus* is differentiated from Micrococcus and other genera’s of the same family. The factors include the Staphylococcus ability. This is used to utilize glucose anaerobically. The cells are slightly smaller than those of *Micrococcus*. The sources of these are the animal body skin.

**Species: Staphylococcus aureus**

Basic habitat of *S. aureus* is the anterior naves. This is observed in the human skin. At the same time, its source is in the respiratory and gastrointestinal tracts. Their size is 0.8 to 0.9 microns. They are found to be having spherical shape. In addition to this, they are found to be non motile and non capsulated. They are gram positive. It can be observed to be in single or in pairs. They grow on nutrient agar. Their growth requires temperature of 37 °C. They are very notorious as they cause pus forming conditions.
The *S. aureus* grows rapidly and produce circular (1-2 mm) endive edge, soft, convex, glistening colonies having a golden yellow pigment. The *S. aureus* can tolerate high concentration of NaCl. Hence, they can be selectively isolated on the nutrient medium containing 7.5 % sodium chloride. It is able to ferment mannitol to organic acid. The *S. aureus* produce the coagulase which is able to clot citrated plasma. It produces the enzymes Catalase, hyaluronidase as well as other virulent factors like enterotoxins, hemolysins, exofoliatin and leucocidins.

**IV.9.2 STREPTOCOCCUS PYOGENES**

Genus: *Streptococcus*

The streptococcus shape is spherical or ovoid. They are non motile, non-sporing and can form chains. Their growth takes place in absence media of native proteins and can produce characteristic hemolytic changes where blood media exists. When the fermentation of carbohydrates takes place, these bacteria produce acids. Some strains of Streptococcus produce exotin and extracellular products. Some of them are found to be anaerobic.

Species: pyogenes

Their size is 0.5 to 0.75 micron. There arrangement is the chain like structure and are having some length. There separation can be done from throat. The matt colonies are showed by them. The glossy type variation will be undergone by them. These are susceptible to some destructive agents such as penicillin and sulfonamides.

**IV.9.3 ESCHERICHIA COLI: ENTEROBACTERIACEAE**

They are Gram negative rods, motile with peritrichate flagella or non motile. They do not form spores. All are found in intestine of man or lower animals.

Genus: Escherichia

This type of genus constitutes *Escherichia coli* and several variants involved.

Species: Escherichia coli

The *Escherichia coli* are a commensally of the human intestine. These are found in the water or soil which are contaminate by fecal matters. They exist in the form of rods and are Gram negative. The size is 2 to 4 microns and has 4 to 8 particrate flagella. They can be grown
in the laboratory media and are sluggishly motile. They are anaerobes and non pathogenic. The strains are found to produce septicemia, gall bladder, appendix and other infections. Such type of pathogens is always recognized in the veterinary field.

**IV.9.4 PSEUDOMONAS AERUGINOSA**

**Genus: Pseudomonas**

*Pseudomonas* is a Greek word (Gr. Pseudo = false, Gr. Monas = a unit) while the word aeruginosa is of Latin origin (L. aeruginosa = full of copper rust i.e. green).

**Species: aeruginosa**

*P. aeruginosa* is Gram-negative short rod bacteria with variable length (1.5-3.0 x 0.5 μm). These are motile and have one or two polar flagella. They are motile by means of one or two polar flagella. The organisms are non-sporulating and non capsulated and few strains possess slime layer up of polysaccharide. The primary habitat of *P. aeruginosa* is human and animal gastro intestinal tract, water, sewage, soil and vegetation. It is physiologically versatile and flourishes as a saprophyte in warm moist situations in the human environment, including sinks, drains, humidifiers, etc. It has been observed that *P. seudomonas* can cause infections when the body resistance is low. Hence they are called as opportunistic pathogens. They are linked with infections caused in the hospitals and due to post burn infections. They can cause infections of middle ear, eyes and urinary tracts. It is associated with diarrhea and pneumonia. Due to drug resistant nature of *P. aeruginosa*, it causes infection in patients receiving long term antibiotic therapy for wounds, burns and cystic fibrosis and other illness. It is found that approximately 25% of burn victims develop infection which frequently leads to fatal septicemia and ten percent are found to be not reacting to the drugs. For this purpose a short duration therapy is preferable.

**IV.10 PROTOZOA**

These parasites always live in cells of the living body. They represent the whole classification of nasty bodies. They always try to make human as the next host. Their entry in the human body causes diarrhea and they will reside in the cells of nervous system. These parasites are as follows, *Entamoeba histolytica, Giardia Lamblia, Logionella, Cryptosopordium* etc.
These may act toxic in certain material contact as like toxic metals in the particular atmosphere and wet surfaces.

IV.11 VIRUSES

The virus is very smaller antibody than single bacteria cell. They hijack other cell organisms to perform disease operations. They spread very speedily.

IV.12 ANTIMICROBIAL ACTIVITY

Concept of Antimicrobial activity

The inhibitory chemicals employed to kill micro-organisms or prevent their growth are called antimicrobial agents for the treatment of diseases. These are classified according to their application and spectrum of activity, as germicides that kill micro-organisms. Whereas microbiostatic agents inhibit the growth of pathogens and enable the leucocytes and other defense mechanism of the host to cope up with static invaders [Ananthnarayan, 2009]. The germicides can exhibit selective toxicity depending on their spectrum of activity. They can act as viricidal (killing viruses), bactericidal (killing bacteria), algicidal (killing algae) or fungicidal (killing fungi). The beginning of modern chemotherapy is said to be due to the efforts of Dr. Paul Ehrlich, who used salvars as arsenic derivative effective against syphilis. Paul Ehrlich used the term chemotherapy for curing the infectious disease without injury to the host’s tissue, known as chemotherapeutic agents such as antibacterial, anti neoplastic, anti viral, anti protocol, anti tubercular and antifungal agents. Later on, Domagk prepared an important chemotherapeutic agent sulfanilamide.

An antimicrobial substance is defined as the substance that ends or prevents the growth of all micro-organisms such as bacteria, fungi or viruses. Consequently, the drugs that kill microbes or prevent the growth of microbes is called antibacterial or antimicrobial drug. The activity related with antimicrobial is called antimicrobial activity.

The antibiotic term is related with antibacterial term. The broader term is also considered for antibiotic which is related with antifungal or antivirus compounds.
IV.13 CLASSIFICATION OF ANTIBACTERIAL AGENTS

The antibacterial agents are classified in following three categories as,
1. Antibiotics and chemically synthesized chemotherapeutic agents
2. Non-antibiotic chemotherapeutic agents (Disinfectants, antiseptics and preservatives)
3. Immunological products.

IV.13.1 ANTIBIOTICS

These play very important role in our everyday life. There are many diseases for which antibiotics are the better solution. The bacteria resistant to other medicines and drugs can be killed easily by using antibiotics. The susceptibility and resistivity of different microorganisms to the antibiotics is very familiar. The different types or groups of bacteria can be studied for understanding the antibacterial activity.

They are produced by micro-organisms. The antibiotics can be prepared either partly or fully by chemical synthesis process. Antibiotics can inhibit the growth of micro-organisms in low concentrations. Antibiotics can be of microbial origin or purely synthetic or semi synthetic. They are classified by manner of biosynthesis or chemical structure. There are also antibiotics as per applicability of it and the susceptible nature of the microbial. The composition of it and the various activities involved is also considered. Structurally, they are classified into different classes as shown below.

IV.13.1.1 CLASSIFICATION OF ANTIBIOTICS ACCORDING TO THEIR CHEMICAL STRUCTURE (Berdy, 1974) [Holt et al., 1994]

1). Carbohydrate-containing antibiotics
   These contain pure sugars, Streptomycin, Amino glycosides, Orthosymycins, N-Glycosides, C-Glycosides, Glycolipids, Nojirimycin, Streptothricin, Vancomycin, Everninomicin, Moenomycin.

2). Macro cyclic lactones
   These constitute the following:
Macrolide antibiotics, Polyene antibiotics, Tetranactin, Erythromycin, Candidin, Ausamycins, Macrotetrolides, Rifamycin

3). Quinones and related antibiotics
Following are quinines and related antibiotics.
Mitomycin, Tetracyclines, Anthracyclines, Naphthoquinones, Benzoquinones, Tetracycline, Adriamycin, Actinorhodin

4). Amino acid and peptide antibiotics
The different types have been given as follows.
Actinomycins, Amino acid derivatives, β-Lactum antibiotics, Peptide antibiotics, Chromopeptides, Depsipeptides, Chelate forming peptides, Cycloserine, Penicillin, Bacteriacin, Valinomycin, Bleomycins

5). Heterocyclic antibiotics containing oxygen
Monessen, Polyether antibiotics

6). Heterocyclic antibiotics containing nitrogen
Polyoxins, Nucleoside antibiotics

7). Aromatic antibiotics
Cycloalkane derivatives, Steroid antibiotics, Cycloheximide, Fusidic acid

8). Aromatic antibiotics
These are classified are classified accordingly as,
Novobiocin, Benzene derivatives, Condensed aromatic antibiotics, Aromatic ether, Chloramphenicol, Griseofulvin.

9). Aliphatic antibiotics
The compounds containing phosphorous fosfomycins synthetic antimicrobial agents include sulfonamides, diamino pyrimidine derivatives, antitubercular compounds, nitrofuran compounds, flucytosine, 4-quinoline antibacterial, imidazole derivatives, etc.

IV.13.2 Non-antibiotics
The second category of antibacterial agents consists of non-antibiotic chemotherapeutic agents which are as follows.

IV.13.2.1 ACIDS AND THEIR DERIVATIVES
If we want to preserve food and pharmaceuticals then we can use organic acids such as benzoic, sorbic, lactic and propionic acids. The salicylic acid has strong antiseptic and germicidal properties as it is a carboxylated phenol. The presence of COOH group can enhance the antiseptic property and to decrease the destructive effect. The benzoic acid is used as an antiseptic and is employed in lotion and ointment. Benzoic acid and salicylic acid are used to control fungi that cause disease such as athlete’s foot. The benzoic acid and sodium benzoate are used as antifungal preservatives. The mandelic acid possesses good bacterio-static and bactericidal properties.

**IV.13.2.2 ALCOHOLS AND RELATED COMPOUNDS**

The various alcohols and their derivatives are used as antiseptics e.g. ethanol and propanol. The antibacterial value of straight chain alcohols increases with an increase in the molecular weight and beyond C8-the activity begins to fall off. The isomeric alcohol can show a drop in activity from primary, secondary to tertiary. The ethanol has extremely numerous applications in pharmacy.

**IV.13.2.3 CHLORINATION AND COMPOUND CONTAINING CHLORINE**

The chlorination is used to disinfect drinking water, swimming pools and for the treatment of effluent from industries. Robert Koch first referred to the bactericidal properties of hypochlorites. We can represent compounds of N-chlorogroup. In this one or more hydrogen atoms are replaced by chlorine atoms.

**IV.13.2.4 IODINE CONTAINING COMPOUNDS**

Iodine containing compounds are used as antiseptic, fungicide and amoebicide. The iodophores are used as disinfectants and antiseptics. The soaps used for surgical scrubs contain iodophores.

**IV.13.2.5 HEAVY METALS**
The antibacterial activities of heavy metals have been studied by many researchers and scientists. The ions of these metals are easily transferable from the electrode to the electrolyte in the solution. Some of these ions form a stable, passive layer which protects the surface from the corrosion attack.

These heavy metals are mercury, gold, copper etc. Hence these metals are used in disinfectant and antiseptic formulations. They are used in disinfectant and antiseptic formulations. In order to cure wounds, it is better to use mercurochrome and methiolate. The Zinc metal powders are used as the antifungal antiseptics. The copper sulfate is used as algaeicides.

IV.13.2.6 OXIDIZING AGENTS

Their value as antiseptics generally depends on the liberation of oxygen and all other organic compounds. The organic compound will act as killer or preventer of microorganisms present in that environment.

IV.13.2.7 DYES

The organic dyes have been used as antibacterial agents. The bactericidal is always exerted by actinides. Also the bactericidal show both gram positive and Gram negative bacteria’s. These dyes act as an inhabitants in some solutions so that growth will be limited up to some limit.

IV.13.2.8 8-HYDROXYQUINOLINES

The 8-hydroxyquinoline or oxen is unique among the isomeric hydroxyl quinolines, for it alone exhibits antimicrobial activity. This attributes to its ability to chelate metals, which the other isomers do not exhibit.

IV.13.2.9 SURFACE ACTIVE AGENTS
The soaps and detergents are used to remove microbes mechanically from the skin surface. Anionic detergents remove microbes mechanically. The cationic detergents have antimicrobial activities and can be used as disinfectants and antiseptics. Most of the bacteria and fungi are killed or removed when in contact.

IV.13.3 IMMUNOLOGICAL PRODUCTS

The certain immunological products such as vaccines and monoclonal antibodies are used to control the diseases as a prophylactic measure.

IV.14 MODE OF ACTION

The antimicrobial drugs interfere chemically with the synthesis of function of vital components of microorganisms, the cellular structure and functions of eukaryotic cells of the human body. These differences provide us with selective toxicity of chemotherapeutic agents against bacteria. The antibacterial drugs either prevent growth or kill microorganisms depending upon the conditions. There are various ways in which these agents exhibit their antimicrobial activity. They may inhibit

1. the synthesis of nucleic acid,
2. the synthesis of cell-wall,
3. the synthesis of protein,
4. the enzymatic activity, and
5. the foliate metabolism

Detoxification of antibacterial

The P-Aminobenzoic acid is a growth factor for micro-organisms and competitively inhibits the bacteriostatic action of sulfonamides. The metabolites identified in man are p-aminobenzyolglucoronide; p-aminohippuric acid, p-acetylaminobenzoic acid. The 8-hydroxyquinoline (oxine) and 4-hydroxyquinoline are excreted as sulfate esters or glucuronides.

IV.15 EVALUATION TECHNIQUES
If we want to screen the antimicrobial activity then following conditions must be fulfilled.

i) During study period, all the conditions must be same.

ii) The test organisms and substance should be in contact with each other

iii) The required and necessary conditions must be fulfilled for the growth of microorganisms.

iv) There should be sterile and aseptic environment throughout the study.

There are various methods to evaluate antimicrobial activity. These are mentioned below.

i) Agar diffusion method

ii) Turbido-metric method

iii) Agar streak method

iv) Serial dilution method

The Agar diffusion method is again divided into

i) Agar ditch method

ii) Agar cup method

iii) Paper disc method

IV.15.1 AGAR CUP METHOD:

The antibacterial activity can be evaluated by this method. This is non automated test method. The in-vitro bacterial susceptibility test can be performed by this method. This gives us inhibitory zones in mm where growth of microorganisms is restricted. The Petri dishes are used for performing tests.

IV.15.2 KIRBY-BAUER DISK DIFFUSION TEST:

The susceptibility of bacteria to antimicrobials can be determined by disk diffusion method. The original disc diffusion method have been modified according to the materials, environmental conditions, solutions under test, the time required for performing the test and applicability to the particular situation [Singh, 2013]. The modification is also related to the own
needs, medial utilization, incubation period, concentration of the inoculums and compounds. When the antibiotic disc was placed in the inoculums, the inhibition zones were observed. The sensitivity and susceptibility depend on the concentration along with resistivity. These can be interpreted by measuring the zone diameter. The co-relation, measurement and interpretation of above mentioned concepts were put forth by Bauer and Kirby in the systematic way [Bauer (1) et al., 1959] [Bauer (2) et al., 1966]. Initially single disc was used. The inhibition zone surrounding the disc may be present or absent but its effect is very important. The most clinical microbiology laboratories utilize the disk diffusion method with variation and modification depending on the conditions. The standardized procedure has also been devised by World Health Organization researcher committee and they named it as Kirby-Bauer disk diffusion test [CLSI, 2006]. The reproduced results have been conformed from different tests.

There is much freedom of change in this method. **Organisms which are pathogenic can be grown on Muller-Hinton Agar in Petri plate.**

The filter paper disks which are impregnated by antimicrobial compound are placed in such Petri plates. The growth of microorganisms is observed from zones formed around the disk. W can measure diameters and can predict the inhibition zone characteristic as well as the the microorganisms information.

**IV.16 ANTIMICROBIAL SUSCEPTIBILITY TESTS**

These tests are important for the invention of new microbial and antibiotics. These tests also give the new sources and causes related with pure water. In practice, agents are commonly used and the laboratory test serves to explain treatment failures and to provide a range of suitable alternative agents. The routine testing also provide up-to-date accumulated data from which information on the most suitable agents for empirical use will be derived, apart from usual routine clinical work. Antimicrobial susceptibility tests are generally used to evaluate the in vitro activity of new agents.

Antimicrobial susceptibility can be reported qualitatively, as sensitive, inter-mediate or resistant.
1. **THE MINIMUM BACTERICIDAL CONCENTRATION (MBC)**

   The concentrations of agents defining the breakpoints’ between the different categories of susceptibility are mainly based on clinical, pharmacological and microbiological considerations. Laboratory staff and clinicians must be aware of the meanings of the categories of susceptibility. The sensitive indicator that the standard dose of the agent is appropriate for treating the patient infected with the strain tested.

2. **INTERMEDIATE**

   This can also be referred to as moderately resistant, moderately susceptible or in determinant. This indicates that the strain can inhibit by larger doses of the agent or may be inhibited in sisters. It is also being regarded as reporting a resistant isolate as susceptible or vice versa. They may indicate intermediate category on patient reports are to be avoided as clinicians find it helpful. In contrast, Laboratory staff should be aware of the meaning and significance of this category of susceptibility.

   Breakpoint concentrations of antimicrobial agents, which are used to separate susceptible from resistant and / or intermediate isolates on the basis of growth or no growth at the selected concentration has been published in several countries.

   Such breakpoints concentration may be used to interpreter, as MIC results test concentrations in breakpoint methods. Or as the relationship of zone sizes to MICs by closely defined methods has been established for agents tested.

**IV.17 TECHNICAL METHODS**

The three principal methods used for susceptibility testing outlined briefly below. There follows a discussion of technical factors relevant to all methods and subsequently detailed consideration of the individual method.

**IV.17.1 DIFFUSION TESTS**
Now a day diffusion tests with agents in paper discs are still the most widely used methods in routine clinical laboratories. They are technically straightforward and relatively very cheap.

**IV.17.2 MINIMUM INHIBITORY CONCENTRATION METHOD**

In this method (MIC), the susceptibility of organisms is to serial twofold dilution of agent in agar or broth and is usually determined. It can be defined as the lowest concentration of the agent that inhibits visible growth. The methods are not widely use for routine testing in clinical laboratories in most countries, although commercial systems with restricted range MICs in micrititre format are frequently used in some methods.

There is unreliable thing as with slowing-growing organisms. Dilution MIC methods are accepted as the standard against which other methods are assessed. In some cases it is necessary to know the minimum concentration of an agent that kills, rather than merely inhibits the growth of a bacterium under consideration. The MBC involved from tubes showing no turbidity onto antimicrobial agent-free media and observing for growths after further incubation.

**IV.17.3 BREAK POINT METHOD**

Break point methods are essentially MIC methods in which only one or two concentrations are tested in a systematic way. The concentration chosen for testing are those equivalents to the breakpoint concentration separating different categories of susceptibility. In recent years such methods have been used routinely as an alternative to disc diffusion method. Breakpoint tests are suitable for automated inoculation and reading of test, and may be combined with identification tests.

**IV.18 FACTOR AFFECTING SUSCEPTIBILITY TESTS**

Many of the effects of technical variation on susceptibility testing are widely known. Following factors are important for the testing the susceptibility.

1. **MEDIUM**

The medium should support the growth of organisms normally tested and should not contain antagonists of antimicrobial activity. For example, thymidyne antagonizes the activity of
sulphonamides and trimethoprim, leading to false reports of resistance to these agents. If the medium is not low in thymidine, lyses horse blood or thymidine phosphorylase should be added to remove free thymidine.

Mono-valent atoms contain caution such as Na+ which increases the activity of backtracking fluidic acid and novobiocin against staphylocci, and of penicillin against protease Sapp. The divalent caution such as Mg$^{2+}$ and Ca$^{2+}$ can reduce the activity of amino glycoside antimicrobials and polymyxins against pseudomonas spp. and of tetracycline against a range of organisms present.

Media specially formulated for susceptibility testing are always recommended. They should not be related to antagonistic to antimicrobial activity and are consistent in their performance. Iso-sensitive agar (oxide) is popular in the UK, and Mueller Hinton agar (various suppliers) is widely used in several other countries.

There have been problems with consistency of batches of Mueller-Hinton agar which has led to the publication of a performance standard. Even with iso zestiest agar, which is produced by a single manufacturer, there have been problems with variation among batches recommends a supplemented Mueller-Hinton Agar medium for testing Haemophilus spp and a supplemented GC agar for Neisseria spp.

2. **THE pH VALUE**

The activity of amino glycosides, microclines, lincosamides and nitrofuration increases with increasing pH value of the solution. Conversely, the activity of tetracycline, fusidic acid and novobiocin increases as the pH of the solution is lowered.

3. **DEPTH OF AGAR MEDIUM**

The plates are considered to be having depth of 3 to 4 mm. With diffusion tests the size of the zone increases as the depth of the medium decreases but this effect is most marked with very thin plates, which should be avoided.

4. **INOCULUMS SIZE**
Increasing inoculums size reduces the susceptibility to agent in both diffusion and dilution tests. Tests of susceptibility to sulphonamides, trimethoprim and beta-lactum agents where resistance is based on beta-lactamase production in stphylo cocci, Haemophilus spp. and Neisseria spp., and methicillin resistance in staphylococci are particularly affected by size.

In most diffusion methods an inoculums size that gives a semi–confluent growth is used. This has the advantage that incorrect inoculants can be readily identified and the test rejected. There is no evidence however that semi-confluent growth gives more reliable results than just confluent growth as used in the NCCLS diffusion methods.

In agar dilution (MIC or breakpoint) methods and inoculums of $10^4$ cfu/inoculums spot is the accepted standard everywhere. This should be increased to $10^6$ cfu/inoculums spot for a methicillin susceptibility test on staphylococci.

This can be avoided by increasing the inoculums to $10^6$ cfu/inoculums spot on agar dilution plates. For broth dilution methods the standard inoculums should be $10^5$ cfu/ml of test broth at the beginning of the incubation period under consideration.

Media containing thymidine should not be used to prepare inoculates for tests on sulphonamides and trimethoprim. We can have inoculants photo-metrically or by standard dilution of broth cultures if the density of such a cultures is reasonably constant.

If there is any doubt about whether particular inoculums preparation procedure yields the requisite density of organisms then it should be checked by performing viable counts on the small number of representative isolates before embracing on a series of MICs test. Inoculants must be used promptly to avoid subsequent changes in their density. Plate should inoculate within 30 min of standardizing the inoculums.

5. PREINCUBATION AND PREDIFFUSION

In diffusion tests, pre-incubation and pre-diffusion decrease the sizes of zones respectively. In practice these effects are avoidable by applying discs soon after plates are inoculated and incubating plate soon after discs are applied.

IV.19 ANTIMICROBIAL AGENTS/DISCS
Modern commercial discs are generally produced to a high standard and problems caused by incorrect content or incorrect agents in discs are very rare. Humidity will cause deterioration of labile agents and many of the problems with discs are related to incorrect handling in the laboratory and instruments. We should store the discs in safety containers and in the dark.

The containers may constitute desiccants. The discs are placed below the 8\(^{0}\)C temperature. The warming of containers is achieved by warming it so that we can condense it. It is necessary to warm all the containers before use.

Problem with humidity also affect pure antimicrobial powders used in testing and similar precaution to those described for disc should be taken powders absorbs water. The amount active substance per weighed amount will be reduced even if the agent is not labile because of the weight of the water absorbed.

**IV.20 INCUBATION**

Incubation of plate is carried at 35 to 37 \(^{0}\)C in air. It is better to use alternative condition only if essential for growth of the organism. Incubation in an atmosphere containing additional carbon dioxide may reduce the pH, which may affect the activity of some agent. Incubation at 30 \(^{0}\)C is usually necessary for methicillin / oxacillin susceptibility test staphylococci but should not be used for other agents a zone size which will be increased.

**READING DIFFUSION RESULTS**

Unless tests are read by an automated system, the reading is somewhere subjective unless end points will be written down available to staff who do these tests.

The diffusion results are usually read by considering at least three readings at same physical conditions, temperature, pressure, humidity etc. The simultaneous measurement is very necessary for the accurate or correct results. If results are not repetitive then they should be discarded. The end points are considered to be check list during the observations. The minority resistant population depends on the end points. In case of broth dilution tests, it is necessary to take the precaution of measure. When the contamination is severe, the end point of particular test is important. The inhibition of growth is considered in the MIC as the lower concentration.
Sometimes there are large zones which are related with colonies indicating that contamination is also large.

**IV.21 PURE CULTURE TECHNIQUES**

Microorganism population can be increased when required by taking the smear in a systematic way and in mixed form. The natural population of microbial can be maintained at different temperatures by considering the prevention measures. We have no much space for the development of microorganisms. The great sterility is observed during the testing procedure. The contamination by external agents should be protected. The atmosphere is the basic block of contamination and increase in population of microorganisms.

The form of a petri dish, with its overlapping lid, is specifically designed to prevent atmospheric contamination. Contamination of tubes and flasks is prevented by closure of their orifices with an appropriate stopper. This has traditionally been a plug of cotton wool, although metal caps or plastic screw caps are now often employed, particularly for test tubes.

The external surface of a culture vessel is, of course, subject to contamination, and the interior of a flask or tube can become contaminated when it is opened to introduce or withdraw material. This danger is minimized by passing the orifice through a flame, immediately after the stopper has been removed and again just before it is replaced.

The inoculum (i.e., the microbial materials used to seed or inoculate a culture vessel) is commonly introduced. There should be rapid sterilization before the using vessels. They can be heated on flame. The pipette is used for transferring the liquid culture. For this purpose, the mouth end of the pipette may be plugged with cotton wool, and the pipette is sterilized in a paper wrapping or in a glass or metal container, which keeps both inner and outer surfaces free of contamination until the time of use.

The risks of accidental contamination may be further reduced by performing transfers in a hood or in a small closed room, the air of which has been specially treated to reduce its microbial content. Special hoods and other precautions may also be necessary to prevent accidental release of the organism if it causes disease, or if it has been constructed using recombinant DNA techniques.

**IV.21.1 ISOLATION OF PURE CULTURES BY PLATING METHODS**
In the plating method, pure cultures can be obtained if we modify it for the study of different bacteria. The solid medium is selected for the purpose of this. In this method we can separate it by mobilizing the particular organism by using a particular nutrient medium, generally agar medium. The colonies are grown on to a colony which will be readily available. We can prefer streaking for this purpose i.e. for growing the cultures. In this method the dipping wire is done by bending it and placing in the plates.

The streaking is accomplished by sterilizing all the materials. The dilution of inoculums is necessary in the progressive manner. This process is continued until the colonies are formed along the large tracks. At that instant, the isolation is performed with the pored plates. The dilutions may be placed by using the petri dished in the sterilized form and then the mixing is performed. It is important that we should make embedding in the agar medium successively.

There are also some drawbacks in the plating method which raises very serious problems. These are no killing of bacteria with faster rate, due to the exposure to the oxygen. The sensitive anaerobe acquires oxygen easily and decreases the rate of killing. So the inhibition zones formed will take longer time to develop. For this purpose it is better to select proper culture conditions or dilution shake culture. The transfer process should be modified. When the dilution process continues after 5 to 10 successive dilution processes, the tubes will be cooled rapidly. The sealing can be done by pouring the sterilized jelly of the petroleum. The paraffin will be poured on the surface. Thus air access is largely prevented. In order to transfer colonies we should remove the surface jelly i.e. paraffin seal by a fine needle. The column formed can be separated into different sections by a pointer or the knife, which is sterilized, and the process of transfer of the colonies takes place.

Many bacteria are killed by even momentary exposure to air; thus successful cultivation of these strict anaerobes, as they are called, requires extra-ordinary measures to exclude at all times even traces of oxygen. Two principal techniques are currently used to culture bacteria in complete absence of oxygen; they are the roll tube and anaerobic glove box techniques. The roll tube procedure developed by Hun gate is used to obtain pure cultures of strict anaerobes by distributing cells in a thin layer of agar on the walls of a test tube, where they develop into isolated colonies. The test contains a few milliliters of molten agar medium that has been reduced chemically to remove dissolved oxygen and is tightly stoppers with a butyl rubber bung
(small but lethal quantities of oxygen diffuse through ordinary rubber). The molten agar is inoculated with appropriate dilutions of the source of bacteria by inserting them through the rubber stoppers with a sterile syringe. The tubes are then laid on their sides in ice and rolled until the agar solidifies in a thin layer on the wall of the tube. After a period of incubation when colonies become visible, the bung is removed and isolated colonies picked from the agar with a needle or capillary tube. Whenever a tube is non stopper entry of air is prevented by continuously passing a stream of O$_2$– free gas (normally CO$_2$ or N$_2$) into the tube. To ensure that some entry of air has not found, it is usual to include in the medium the redox dye resazurine, which changes from colorless to red at a midpoint $E_h$ of $–0.042\,\text{V}$.

Strict anaerobes may also be isolated using conventional streak plate techniques if all procedures are done within a glove box that encloses a reducing atmosphere.

In isolating from a mixed natural population it is often possible, provided one’s technique is good, to prepare a first plate, or dilution shake or roll tube series, in which many of the colonies that developed are well separated from one another. Although this is often done, a culture so isolated may be far from pure. There is a significant probability that any particular colony was initiated by two similar microorganisms seeded together on the plate to produce a mixed colony. Furthermore, because microorganisms vary greatly in their nutritional requirements, no single medium and set of growth conditions will permit the growth of all the microorganisms present in a natural population.

There is very small transfer of colonies in a fraction. The formation of colonies of microorganisms on the medium takes place. Hence, for every visible colony on a first plate, there may be thousands of other microorganisms that were also deposited on the agar surface but that failed to give microscopically visible growth, although they may still be viable. The probability is high that some of these organisms will be picked up and carried over when a transfer is made. One should never pick from a first plate for the preparation of a pure culture.

Not all microorganisms able to grow on solid media necessarily give rise to well-isolated colonies. Certain motile flagellated bacteria (Proteus, Pseudomonas, for example) can rapidly spread over the slightly moist surface of a freshly poured plate. This can be prevented by the use of plated with well-dried surfaces, on which the cells are immobilized Spirochetes and organisms that show gliding movement (e.g., myxobacteria, many cyanobacteria) can move over or through
an agar gel, even when its surface is well dried. In such cases, the movement of the organisms in question may be an aid to their purification, since they can move away from other kinds of microorganisms immobilized on the agar. Thus, purification can often be achieved by allowing migration to occur and transferring repeatedly to fresh plates from the advancing edge of the migrating population.

The incorporation into the medium of selectively inhibitory substances is also sometimes helpful in making isolations from nature. Because of their biological specificity, certain antibiotics are particularly useful in this respect. Bacteria vary greatly in their sensitivity to the antibiotic penicillin which can consequently be used at low concentrations. Penicillin is generally toxic for prokaryotic organisms but not for eukaryotic ones. It is thus a very useful agent for the purification of protozoa, fungi and eukaryotic algae that are contaminated by bacteria. Conversely, prokaryotic organisms are insensitive to polynye antibiotics such as nystatin, which are generally toxic for the eukaryotic organisms. The incorporation of this kind of antibiotic into the isolation medium can sometimes be used to advantage in the purification of bacteria heavily contaminated by the fungi or amoebae. Many other variations on the theme of selective toxicity can be used to facilitate isolations by the plating method.

**IV.21.2 ISOLATION OF PURE CULTURES IN LIQUID MEDIA**

Plating methods are in general satisfactory for the isolation of bacteria and fungi because the great majority of the representatives of these groups can grow well on solid media.

Many protozoa and algae are also cultivable only in a liquid medium. Although plating methods for the isolation of viruses have been greatly extended in recent years. In the case of viruses, of course, a pure culture is never obtainable since these organisms are obligate intracellular parasites; a two – membered culture, consisting of a specific virus and its biological host, represents the goal of purification for this microbial group.

There is a dilution method for the isolation in the liquid medium. We have inoculums in the form of sterile medium. The medium in the form of tubes can be isolated by the successive dilution. The microbe solution in the different tubes is poured in the separate tubes. The sealing procedure follows the statistical analysis. The probability theories help to determine the size in
the fraction up to the 0.00002. Such probability is resulted from the single organism. This can be increased with in inoculums. For this purpose large numbers of tubes are selected for no growth.

This method is used for the isolation of mixed population. It is the predominant member family. This results in to the drawback of the method. If there involve large population of microorganisms then it fails. It was found that the method have limitations.

In the case of large microorganisms purification involves the capture of a single individual in a fine capillary pipette and the subsequent transfer of this individual through several washings in relatively large volumes of sterile medium to eliminate microbial contaminants of smaller size. The successive operations can be performed manually, with control by direct microscopic observation at a relatively low magnification such as that provided by a dissecting microscope.

The technique of the capillary pipette can no longer be applied if the organism that one wishes to isolate is so small that it cannot be readily observed at a magnification of 100 times or less, because one cannot achieve the necessary fineness of control to manipulate a capillary pipette directly at higher magnifications. In this event, a mechanical device known as a micromanipulator must be used in conjunction with specially prepared, very fine glass operating instruments. The essential purpose of a micromanipulator is to gear down manual control, so that very slight and precisely controlled movements of the operating instruments can be effected in a small operating area (a micro drop) under continuous microscopic observation at high magnifications (500 to 1,000 X).

### IV.21.3 TWO MEMBER CULTURES

The goal of isolation is normally to obtain a pure culture. However, there are certain situations where this cannot be achieved or where achievement is so difficult as to be impractical. Under such circumstances, the alternative is to obtain the next best degree of purification in the shape of a two-member culture, which contains only two kinds of microorganisms. As already mentioned, a two-member culture is in principle the only possible way to maintain viruses, since these organisms are all obligate intracellular parasites of cellular organisms. Obligate intracellular parasitism is also characteristic of several groups of cellular
microorganisms. In all these instances a two-member culture represents the nearest approach to cultivation under controlled laboratory conditions that can be achieved.

Many of the protozoa, which feed in nature on smaller microorganisms, are also most easily maintained in the laboratory as two-member cultures in association with their smaller microbial prey. This is true, for examples, of ciliates, amebas, and slime molds. In such instances the associations is probably never an obligate one, since careful nutritional studies on a few representatives of these groups have shown that they can be grown in pure culture: however, the nutritional requirements of protozoa are often extremely complex, so that the preparation of media for the maintenance of pure cultures is both difficult and laborious. For purposes of routine maintenance and also for many experimental purposes, two-member cultures are satisfactory.

The establishment of a two member culture is an operation that is conducted in two phases. First, it is necessary to establish a pure culture of the food organism, the host in the cases of obligate intracellular parasites, the prey in the case of protozoa. Once this has been achieved, the parasite or predator can be isolated by any one of a variety of methods (plating on solid media in the presence of the food organism, dilution in liquid medium, single-cell isolation) and introduced into the pure culture of the food organism.

The successful maintenance of two-member cultured requires considerable art because a reasonably stable biological balance between the two components is essential. The medium must be one that permits sufficient growth of the food organism to meet the needs of the parasite or predator but should not be so rich that the food organism can outgrow its associate or produce metabolic products that are deleterious to it.

IV.22 STERILIZATION: THEORY AND PRACTICE

In this process, we can material or liquid to be free from external agents such as microorganism development. This process can be achieved by different methods. The plating method can be used during sterilization. In this method the survivors can be detected if we form different colonies. The deaths of population of organisms are controlled by lethal agent use. There will be decrease proportionally if the sensitive operations result. The actual time of death of the organisms can measure by knowing the probability. The direct proportionality gives the
variation straight forward. The initial population survival is determined from the death rate. Also the size of the initial population is important in the determination which is related to the sterilization conditions and parameters.

In the practice of sterilization the microbial population to be destroyed is almost always a mixed one. Since microorganisms differ widely in their resistance to lethal agents, the significant factor become the initial population size and the death rate of the most resistant members of the mixed population. These are almost always the highly resistant endo-spores of certain bacteria. Consequently spore suspensions of known resistance are the object commonly used to assess the reliability of sterilization methods.

Taking into account the kinetics of microbial death, we can formulate the practical goal of sterilization by a lethal agent in a slightly more refined way: the probability that the object treated contains even one survivor should be infinitesimally small. For example, if we wish to sterilize a liter of a culture medium, this goal will be achieved for all practical purposes if the treatment is one that will leave no more than one survivor in $10^6$ liters. Under such circumstances, the probability of failure is very small indeed. Procedures of routine sterilization are always designed to provide a very wide margin of safety.

IV.23 ANTIMICROBIAL ACTIVITY OF METALS AND ALLOYS

To be potable, water must be more or less free from turbidity, odor, and color, taste, well aerated. It must be free from pathogenic microorganisms and harmful chemicals. The various methods of water purifications are in use which depends on the amount and character of water as flocculation, filtration, sedimentation and disinfection.

There is a considerable research in the field of water purification materials used in purifiers, filters and chemicals used as antimicrobials [Bhagwat et al., 2013]. The traditional methods of water purification have many drawbacks, such as corrosion of containers used, coating dissolution, pitting and ageing effects and cost. Some steels have corrosion problem which leads to increase in impurities and cost. The plastic materials though have handling, transport and cost advantage, but they impart a smell due to physical conditions or dissolution of materials of containers.
The antimicrobial activity of some metals, alloys and chemicals have been reported. Yokota et al. showed that stainless steel having high corrosion resistance and having specific constituents with specific preparation of alloy acts as antimicrobial material. Many researchers had worked on different materials which showed antibacterial property and superior corrosion resistance. A stainless steel selected from the group consisting of austenitic (10-35% by weight of Cr), ferritic (10-50% by weight of Cr) and martensitic (10-19% by weight of Cr) stainless steel, said stainless steel having antibacterial properties. They found that stainless steel having superior antibacterial properties and also superior corrosion resistance could be obtained [Difco, 1984]. The research on stainless steels had further showed that the antibacterial property is dependent on basic constituents used in the steel. A peculiar steel showed antibacterial activity for \textit{P. gingivalis} bacteria. The implant-related infections can be reduced in the orthodontic treatments are possible [Zhang, 2013]. The antibacterial property of silver nano-particles against \textit{P. aeruginosa} had been reported by Amiruthusini et al. [Amirulhusni, 2012]. The research on drugs coated with against \textit{B. Cereus}, \textit{L. monocytogenes} and \textit{S. aureus} showed antibacterial property. It was concluded that food poisoning can be avoided by coating method. The different fungal isolates were gown and tests were carried out for the different samples.

The study performed by Drew showed that we can attain perfection and find out results. The bacterium was found to be methillin resistant [Drew et al., 1972]. The street food contamination detection study was performed. The fecal \textit{coliforms} and \textit{B. aureus} affection was detected [World, 2007].

Maraging steels of different compositions had been used in different applications. Their properties had attracted to find out the openings of utility [Grum, 2006] [Shinde (1) et al., 2013] [Shinde (2) et al., 2013] [Shinde (4), 2013] [Shinde (6) et al., 2013]. It seemed that there is need to study antimicrobial activity of maraging steel in the drinking water of Manchar town by using modified Kirby Buyer Method [Jiang et al., 2007] [Sheretz et al., 1989].

**IV.24 BASIC INFORMATION ABOUT CHROMIUM IN DRINKING WATER**

The chromium is generally used for changing properties of any base metal, the application required and the effects under the study involved. It always gives shining to the material. In the decorative metal industry, it plays very important and advantageous role. In order
to avoid corrosion and protect the base metal from the acidic environment, it is coated on the materials. The atmospheric effects on the materials can be avoided by using iron-chromium, copper chromium and silver-chromium alloys.

If there are utensils made of materials containing chromium then it is better to consider the level of utility. Such utensils are used for drinking water storage purposes for longer times. Hence there is possibility of excess dissolution of chromium along with other constituents. Internationally the drinking water standard for total chromium has been decided to be as .1 mg/liter. There two types of chromium’s as chromium-6 and chromium-3.

The chromium with valence three is a dietary element in the food viz. vegetables, meat, some grains and yeast. It has been detected in the rocks, soil, volcanic dust and some plants. It is also observed in some natural water sources. If the chromium is taken in excess than certain levels then some diseases may get linked with such person, e.g. dermatitis or skin diseases [Basic, 2000].

Many stainless steels consist of chromium metal. Accordingly, the steels are also classified on the percentage of chromium mixed, the process of mixing, the characteristics of particular steel alloy formed and use or applicability of the steel material. The utensils used in our everyday life consist of chromium metal or they are surface coated. In order to obtain many advantages of chromium, it is better to select the best process of deposition and the environmental factors.

In many developed countries, a definite standard for maximum level of the chromium metal use and its dissolution in the water have been decided to avoid the misuse of chromium metal as well as to increase the life expectancy.

Thus by considering standards, we can deposit chromium on maraging steel samples and can test antimicrobial activity of treated samples. The effects of chromium deposition are very important in our everyday life, so we selected chromium deposition for our novel research study.