FOOD & PHARMACEUTICAL MICROBILOGY
(PHARMACEUTICS-VII)
B.PHARM

UNIT-II (STUDY MATERIAL)

B.PHARM

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INTRODUCTION

In a rather broader perspective the 'bacteria' are markedly distinguished by their inherent extreme metabolic diversity; whereas, a few of them may conveniently sustain themselves exclusively on 'inorganic substances by strategically making use of such specific pathways which are practically absent amongst the plant as well as animal kingdoms. Based upon the aforesaid statement of facts one may individually explore and exploit the various cardinal factor(s) that essentially govern the *nutrition, cultivation* (*growth*), and *isolation* of bacteria,

BACTERIA:-

The nutrition, cultivation (growth), and isolation of bacteria shall be dealt with in the sections That follows:-

Nutrition of Microorganisms (Bacteria)

Interestingly, the microbial cell represents an extremely complex entity, which is essentially Comprised of approximately 70% of by its weight as water, and the remaining 30% by its weight as thesolid components. Besides, the *two* major gaseous constituents *viz*., oxygen (O_2) and hydrogen (H_2) the microbial cell predominantly consists of four other major elements, namely: Carbon (C), nitroge(N), sulphur (S), and phosphorus (P). In fact, the six aforesaid constituents almost account for 95% of the ensuing cellular dry weight. The various other elements that also present but in relatively much lesser quantum are : Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+}, Cu^{2+}, Fe^{3+} and Mo^{4+}. Based on these critical observations and findings one may infer that the microorganisms significantly require an exceptionally large number of elements for its adequate survival as well as growth (*i.e.*, cultivation).The following displays the various chemical composition of an *Escherichia coli* cell.

It has been amply proved and established that carbon represents an integral component of almost all organic cell material ; and, hence, constitutes practically half of the ensuing dry cell weight. Nitrogen is more or less largely confined to the proteins, coenzymes, and the nucleic acids (DNA, RNA). Sulphur is a vital component of proteins and coenzymes; whereas, phosphorus designates as the major component of the nucleic acids. It is, however, pertinent to mention here that as to date it is not possible to ascertain the precise Requirement of various elements *viz.* C, N, S and O, by virtue of the fact that most bacteria predominantly differ with regard to the actual chemical form wherein these elements are invariably consumed as nutrients.

Cultivation (Growth) of Bacteria

The cultivation (growth) of bacteria may be defined, as — 'a systematic progressive increase in the cellular components'. Nevertheless, an appreciable enhancement in 'mass' exclusively may not always reflect the element of growth because bacteria at certain specific instances may accumulate enough mass without a corresponding increment in the actual cell number. In the latest scenario the terms. 'Balanced growth' has been introduced which essentially draws a line between the so called 'orderly growth' and the 'disorderly growth'. Campbell defined 'balanced growth' as — 'the two-fold increase of each biochemical unit of the cells very much within the prevailing time period by a single division without having a

Slightest change in the rate of growth'. However, one may accomplish theoretically cultures with a 'Balanced growth 'having a more or less stable and constant chemical composition, but it is rather next to impossible to achieve this. Following are some of the cardinal aspects of Cultivation of bacteria, such as:

Binary Fission

It has been established beyond any reasonable doubt that the most abundantly available means of Bacterial cultivation (reproduction) is binary fission, that is, one specific cell undergoes division to give rise to the formation of two cells.

Isolation of bacteria:-

isolation refers to the separation of a strain from a natural, mixed population of living microbes, as present in the environment, for example in water or soil, or from living beings with skin flora, oral flora or gut flora, in order to identify the microbe(s) of interest. Historically, the laboratory techniques of isolation first developed in the field of bacteriology and parasitological (during the 19th century), before those in virology during the 20th century. Methods of microbial isolation have drastically changed over the past 50 years, from a labor perspective with increasing mechanization, and in regard to the technology involved, and hence speed and accuracy.

General techniques:-

In order to isolate a microbe from a natural, mixed population of living microbes, as present in the environment, for example in water or soil flora, or from living beings with skin flora, oral flora or gut flora, one has to separate it from the mix. This can be achieved in two ways;

Traditionally microbes have been cultured in order to identify the microbe(s) of interest based on its growth characteristics. Depending on the expected density and viability of microbes present in a liquid sample, physical methods to increase the gradient as for example serial dilution or centrifugation may be chosen. In order to isolate organisms in materials with high microbial content, such as sewage, soil or stool, serial dilutions will increase the chance of separating a mixture.

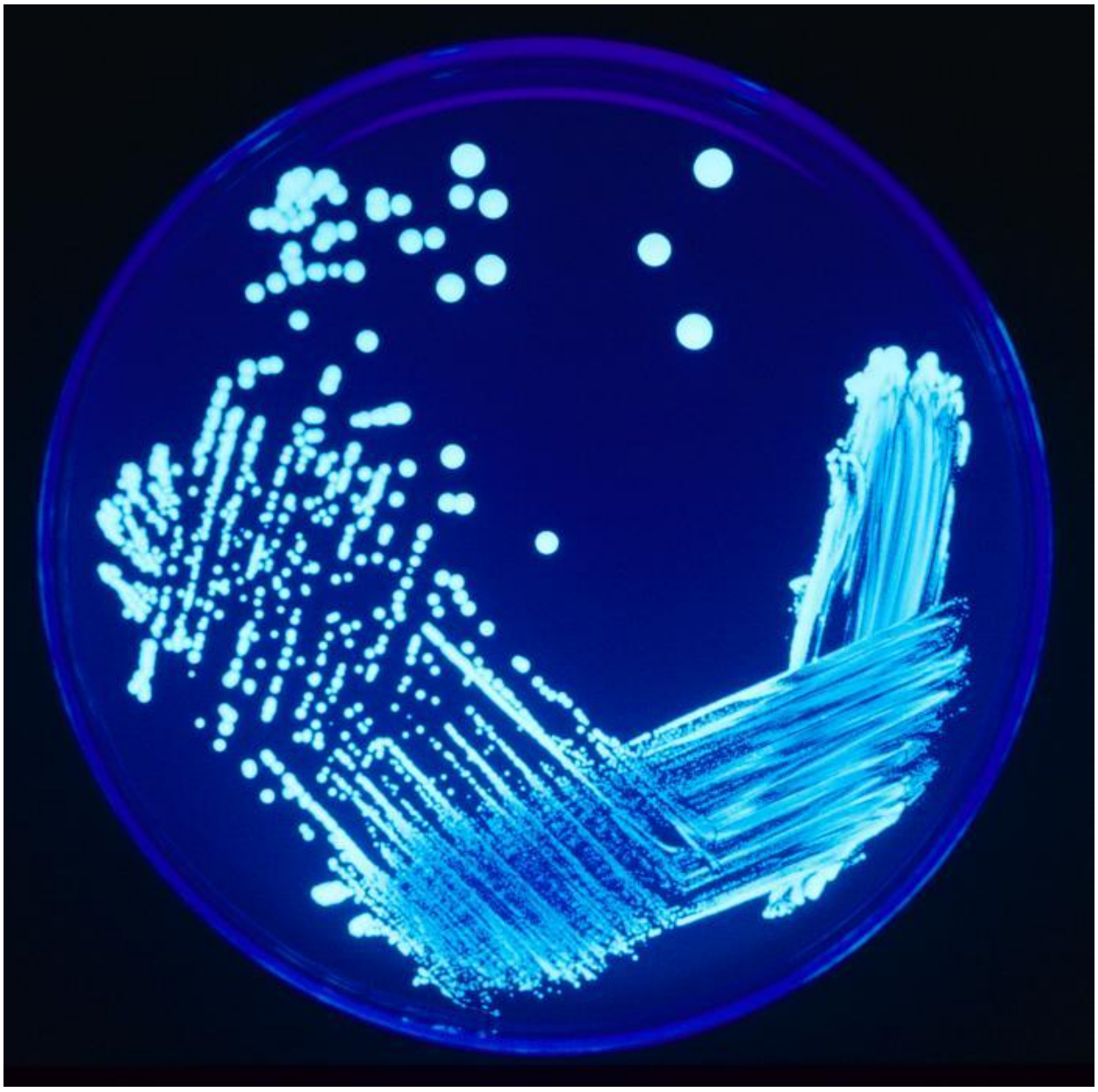
In a liquid medium with few or no expected organisms, from an area that is normally sterile (such as CSF, blood inside the circulatory system) centrifugation, decanting the supernatant and using only the sediment will increase the chance to grow and isolate bacteria or the usually cell-associated viruses.

If one expects or looks for a particularly fastidious organism, the microbiological culture and isolation techniques will have to be geared towards that microbe. For example, a bacterium that dies when exposed to air, can only be isolated if the sample is carried and processed under airless or anaerobic conditions. A bacterium that dies when exposed to room temperature (thermophilic) requires a pre-warmed transport container, and a microbe that dries and dies when carried on a cotton swab will need a viral transport medium before it can be cultured successfully.

More recently, microbes have been isolated without culturing them. Samples are inoculated into microtiter plates or cartridges extracting their particular genetic material (DNA or RNA) which can be used to identifying them.

Streaking method:-

In microbiology, **streaking** is a technique used to isolate a pure strain from a single species of microorganism, often bacteria. Samples can then be taken from the resulting colonies and a microbiological culture can be grown on a new plate so that the organism can be identified, studied, or tested. The modern streak plate method has progressed from the efforts of Robert Koch and other microbiologists to obtain microbiological cultures of bacteria in order to study them. The dilution or isolation by streaking method was first developed by Loeffler and Gaffky in Koch's laboratory, which involves the dilution of bacteria by systematically streaking them over the exterior of the agar in a petri dish to obtain isolated colonies which will then grow into quantity of cells, or isolated colonies. If the agar surface grows microorganisms which are all genetically same, the culture is then considered as a microbiological culture.



Techniques: - Streaking is rapid and ideally a simple process of isolation dilution. The technique is done by diluting a comparatively large concentration of bacteria to a smaller concentration. The decrease of bacteria should show that colonies are sufficiently spread apart to effect the separation of the different types of microbes. Streaking is done using a sterile tool, such as a cotton swab or commonly an inoculation loop. Aseptic techniques are used to maintain microbiological cultures and to prevent contamination of the growth medium.There are many different types of methods used to streak a plate. Picking a technique is a matter of individual preference and can also depend on how large the number of microbes the sample contains.

The three-phase streaking pattern, known as the T-Streak, is recommended for beginners.The streaking is done using a sterile tool, such as a cotton swab or commonly an inoculation. The inoculation loop is first sterilized by passing it through a flame. When the loop is cool, it is dipped into an inoculums such as a broth or patient specimen containing many species of bacteria. The inoculation loop is then dragged across the surface of the agar back and forth in a zigzag motion until approximately 30% of the plate has been covered. The loop then is re-sterilized and the plate is turned 90 degrees. Starting in the previously streaked section, the loop is dragged through it two to three times continuing the zigzag pattern. The procedure is then repeated once more being cautious to not touch the previously streaked sectors. Each time the loop gathers fewer and fewer bacteria until it gathers just single bacterial cells that can grow into a colony. The plate should show the heaviest growth in the first section. The second section will have less growth and a few isolated colonies, while the final section will have the least amount of growth and many isolated colonies.

Growth medium:- The sample is spread across one quadrant of a Petri dish containing a growth medium. Bacteria need different nutrients to grow. This includes water, a source of energy, sources of carbon, sulfur, nitrogen, phosphorus, certain minerals, and other vitamins and growth factors. A very common type of media used in microbiology labs is known as agar, a gelatinous substance derived from seaweed.

Incubation: - Dependent on the strain, the plate may then be incubated, usually for 24 to 36 hours, to allow the bacteria to reproduce. At the end of incubation there should be enough bacteria to form visible colonies in the areas touched by the inoculation loop. From these mixed colonies, single bacterial or fungal species can be identified based on their morphological (size/shape/colour) differences, and then sub-cultured to a new media plate to yield a pure culture for further analysis.

Importance: - Bacteria exist in water, soil and food, on skin, and intestinal tract normal flora. The assortment of microbes that exist in the environment and on human bodies is enormous. The human body has billions of bacteria which creates the normal flora fighting against the invading pathogens. Bacteria frequently occur in mixed populations. It is very rare to find a single occurring species of bacteria. To be able to study the cultural, morphological, and physiological characteristics of an individual species, it is vital that the bacteria be divided from the other species that generally originate in the environment. This is important in determining a bacterium in a clinical sample. When the bacteria is streaked and isolated, the causative agent of a bacterial disease can be identified

Control of microbial Contamination during manufacture

Contamination control is the generic term for all activities aiming to control the existence, growth and proliferation of contamination in certain areas. Contamination control may refer to the atmosphere as well as to surfaces, to particulate matter as well as to microbes and to contamination prevention as well as to decontamination.

Function:-

The aim of all contamination control activities is to permanently ensure a sufficient level of cleanliness in controlled environments. This is accomplished by maintaining, reducing or eradicating viable and non-viable contamination for either sanitary purposes or in order to maintain an efficient rate of production.

Flooring in a material handling area at the pharmaceutical company Lille, France.

Usage:-

One of the most common environments that incorporate contamination control into its standards protocol is the clean room. There are many preventive procedures in place within a clean room environment. They include subjecting clean room staff to strict clothing regulations, and there is often a gowning room where the staff can change clothes under sterile conditions so as to prevent any particulates from entering from the outside environment. Certain areas in the clean room have more stringent measures than others: packaging areas,

corridors, gowning rooms and transfer hatches incorporate strict contamination control measures in order to maintain clean room standards.

Contamination control is also an important asset for industrial laboratories in the pharmaceutical and life science sectors. Other places of use include automotive paint shops, entrances to industrial kitchens and food service providers, many manufacturing areas, and electronic component assembly areas.

More recently, effective contamination control has been a concern for laboratories and other sensitive environments as a bio-securitycrisis management measure. Some banks and insurance companies use contamination control products as part of their disaster management protocols. Preventive measures are devised as preparation for combating potential pandemics or the proliferation of biohazards in any potential terrorist attack.

Types of contamination control:-

Beside particulate matter such as ions and molecules, the most common types of contamination are: People- Hair, fibre particles from bodies and clothes also poor hygiene Environment- Dust particles, contaminated air, work surfaces, gases, movement ceilings, walls and floors Materials- Micro organisms on packaging, packaging also creates particles , fibres , dust. Equipment- Moving parts shavings drive belts. Buildings- Paint flaking, rusty pipe work , poorly maintained surfaces. Water- Micro organisms grow in water. equipment not cleaned correctly left in a damp condition, spills not mopped up properly etc. Many types organisms are potentially detrimental to processes in a critical environment. Seven of the most common contaminants are:

- *Aspergillus niger*
- *Burkholderia cepacia*
- *Clostridium difficile*
- *Escherichia coli*
- Methicillin Resistant *Staphylococcus aureus* (MRSA)
- *Pseudomonas aeruginosa*
- *Salmonella enteritidis*

Effect of contamination:-

Contamination poses a significant risk to technical processes, experiments or production activities, as well as to the individuals involved. Unguarded proliferation of contamination can quickly lead to product damage, yield reduction, product recalls and other outcomes highly detrimental to business. Products in a range of industries are recalled due to ineffective contamination control systems.

Based on this evidence it could be argued that many businesses are not adequately protecting themselves from the harmful effects of contamination, and many products in many industries are being recalled due to unsafe manufacturing processes.

Types of contamination control:-

Body movement causes contamination, and protective clothing such as hats, cleanroom suits and face masks are accordingly basic items of contamination control. Apart from people, another common way for contamination to enter is on the wheels of trolleys used to transport equipment.

To prevent airborne contamination, high-efficiency particulate air (HEPA) filters, airlocks and cleanroom suits are used. HEPA filtration systems used in the medical sector incorporate high-energy ultraviolet light units to kill the live bacteria and viruses trapped by the filter media. These measures restrict the number of particulates within the atmosphere and inhibit the growth of those that are viable.

Copper-alloy surfaces have intrinsic properties which effectively and quickly destroy microbes and they are being installed in healthcare facilities and in a subway transit system as a protective public health measure in addition to regular cleaning. The United States Environmental Protection Agency (EPA) has approved the registration of 355 different antibacterial that kill *E. coli* O157:H7, *methicillin*-resistant *Staphylococcus aureus* (*MRSA*), *Staphylococcus*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa.* The EPA has determined that when cleaned regularly, these copper alloy surfaces:

- Continuously reduce bacterial contamination, achieving 99.9% reduction within two hours of exposure;
- Kill greater than 99.9% of Gram-negative and Gram-positive bacteria within two hours of exposure;
- Deliver continuous and ongoing antibacterial action, remaining effective in killing more than 99.9% of the bacteria within two hours;
- Kill more than 99.9% of the bacteria within two hours, and continue to kill 99% of the bacteria even after repeated contamination;
- Help inhibit the buildup and growth of bacteria within two hours of exposure between routine cleaning and sanitizing steps.

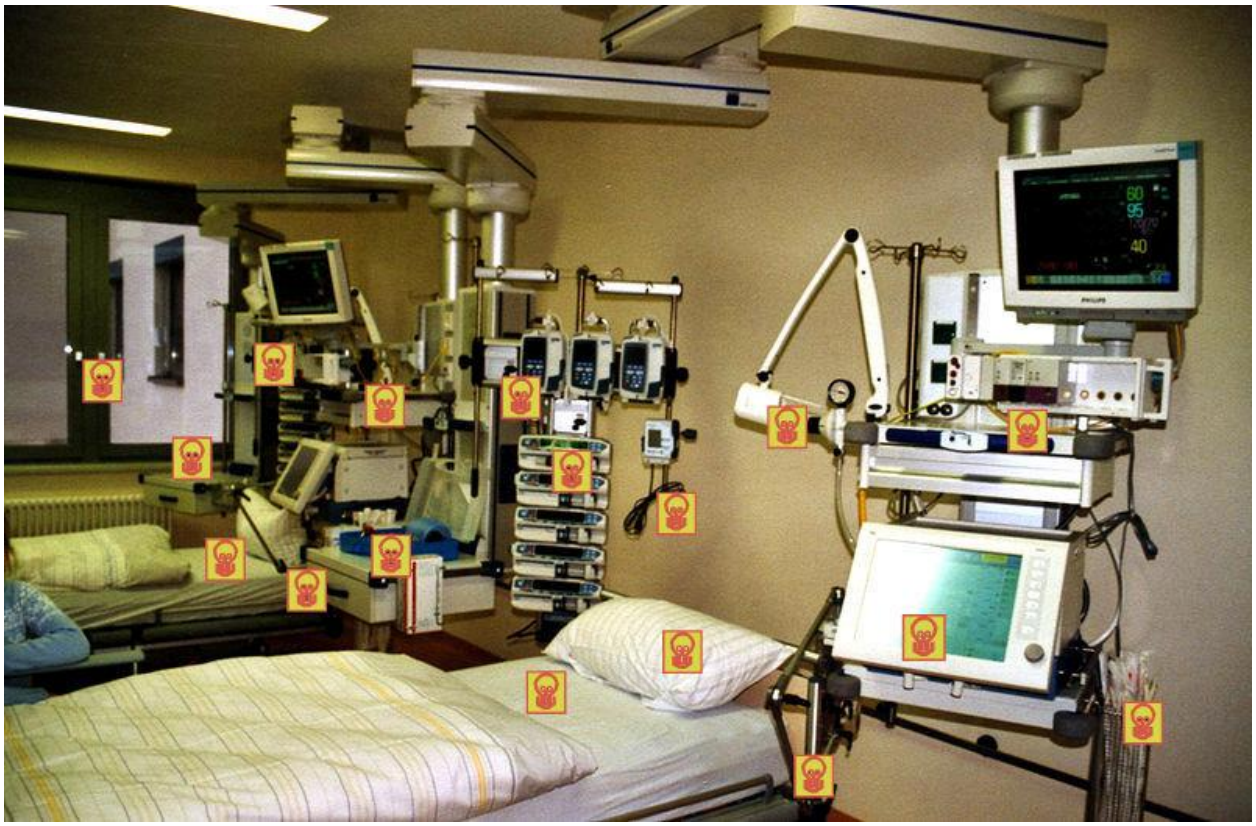
As a contamination control measure, EPA has approved a long list of antimicrobial copper products "with public health benefits" made from these copper alloys, such as bedrails, handrails, over-bed tables, sinks, faucets, door knobs, toilet hardware, computer keyboards, health club equipment, shopping cart handles, etc. (For a comprehensive list of products, see: Antimicrobial copper-alloy touch surfaces # Approved products).

Nosocomial infection & their control

Hospital-acquired infection (**HAI**) — also known as **nosocomial infection** — is an infection that is contracted from the environment or staff of a healthcare facility. It can be spread in the hospital environment, nursing home environment, rehabilitation facility, clinic, or other clinical settings. Infection is spread to the susceptible patient in the clinical setting by a number of means. Health care staff can spread infection, in addition to contaminated equipment, bed linens, or air droplets. The infection can originate from the outside

environment, another infected patient, staff that may be infected, or in some cases, the source of the infection cannot be determined. In some cases the microorganism originates from the patient's own skin microbiota, becoming opportunistic after surgery or other procedures that compromise the protective skin barrier. Though the patient may have contracted the infection from their own skin, the infection is still considered nosocomial since it develops in the health care setting.

In the United States, the Centers for Disease Control and Prevention estimated roughly 1.7 million hospital-associated infections, from all types of microorganisms, including bacteria and fungi combined, cause or contribute to 99,000 deaths each year. In Europe, where hospital surveys have been conducted, the category of gram-negative infections are estimated to account for two-thirds of the 25,000 deaths each year. Nosocomial infections can cause severe pneumonia and infections of the urinary tract, bloodstream and other parts of the body. Many types are difficult to treat with antibiotics. In addition, antibiotic resistance can complicate treatment.



Types:-

- Hospital-acquired pneumonia Ventilator-associated pneumonia
- Urinary tract infection
- Gastroenteritis
- Puerperal fever

Organisms

- *Staphylococcus aureus*
- Methicillin resistant *Staphylococcus aureus*
- *Candida albicans*
- *Pseudomonas aeruginosa*
- *Acinetobacter baumannii*
- *Stenotrophomonas maltophilia*
- *Clostridium difficile*
- *Escherichia coli*
- Tuberculosis
- Vancomycin-resistant *Enterococcus*
- Legionnaires' disease

Cause:-

Transmission

Main routes of transmission

Route	Description
Contact transmission	The most important and frequent mode of transmission of nosocomial infections is by direct contact.
Droplet transmission	Transmission occurs when droplets containing microbes from the infected person are propelled a short distance through the air and deposited on the patient's body; droplets are generated from the source person mainly by coughing, sneezing, and talking, and during the performance of certain procedures, such as bronchoscopy.
Airborne transmission	Dissemination can be either airborne droplet nuclei (small-particle residue {5 μm or smaller in size} of evaporated droplets containing microorganisms that remain suspended in the air for long periods of time) or dust particles containing the infectious agent. Microorganisms carried in this manner can be dispersed widely by air currents and may become inhaled by a susceptible host within the same room or over a longer distance from the source patient, depending on environmental factors; therefore, special air-handling and ventilation are required to prevent airborne transmission. Microorganisms transmitted by airborne

	transmission include *Legionella*, *Mycobacterium tuberculosis* and the rubella and varicella viruses.
Common vehicle transmission	This applies to microorganisms transmitted to the host by contaminated items, such as food, water, medications, devices, and equipment.
Vector borne transmission	This occurs when vectors such as mosquitoes, flies, rats, and other vermin transmit microorganisms.

Contact transmission is divided into two subgroups: direct-contact transmission and indirect-contact transmission.

Routes of contact transmission

Route	Description
Direct-contact transmission	This involves a direct body surface-to-body surface contact and physical transfer of microorganisms between a susceptible host and an infected or colonized person, such as when a person turns a patient, gives a patient a bath, or performs other patient-care activities that require direct personal contact. Direct-contact transmission also can occur between two patients, with one serving as the source of the infectious microorganisms and the other as a susceptible host.
Indirect-contact transmission	This involves contact of a susceptible host with a contaminated intermediate object, usually inanimate, such as contaminated instruments, needles, or dressings, or contaminated gloves that are not changed between patients. In addition, the improper use of saline flush syringes, vials, and bags has been implicated in disease transmission in the US, even when healthcare workers had access to gloves, disposable needles, intravenous devices, and flushes

Prevention:-

Controlling nosocomial infection is to implement QA/QC measures to the health care sectors, and evidence-based management can be a feasible approach. For those with ventilator-associated or hospital-acquired pneumonia, controlling and monitoring hospital indoor air quality needs to be on agenda in management, whereas for nosocomial rotavirus infection, a hand hygiene protocol has to be enforced.

To reduce HAIs, the state of Maryland implemented the Maryland Hospital-Acquired Conditions Program that provides financial rewards and penalties for individual hospitals based on their ability to avoid HAIs. An adaptation of the Centers for Medicare & Medicaid Services payment policy causes poor-performing hospitals to lose up to 3% of their inpatient revenues, whereas hospitals that are able to avoid HAIs can earn up to 3% in rewards.

Sterilization:-

Sterilization goes further than just sanitizing. It kills all microorganisms on equipment and surfaces through exposure to chemicals, ionizing radiation, dry heat, or steam under pressure.

Isolation:-

Isolation is the implementation of isolating precautions designed to prevent transmission of microorganisms by common routes in hospitals. (Universal precautions and Transmission-based precautions.) Because agent and host factors are more difficult to control, interruption of transfer of microorganisms is directed primarily at transmission for example isolation of infectious cases in special hospitals and isolation of patient with infected wounds in special rooms also isolation of joint transplantation patients on specific rooms.

Hand washing:-

Hand washing frequently is called the single most important measure to reduce the risks of transmitting skin microorganisms from one person to another or from one site to another on the same patient. Washing hands as promptly and thoroughly as possible between patient contacts and after contact with blood, body fluids, secretions, excretions, and equipment or articles contaminated by them is an important component of infection control and isolation precautions. The spread of nosocomial infections, among immune compromised patients is connected with health care workers' hand contamination in almost 40% of cases, and is a challenging problem in the modern hospitals

Gloves

In addition to hand washing, gloves play an important role in reducing the risks of transmission of microorganisms. Gloves are worn for three important reasons in hospitals. First, they are worn to provide a protective barrier for personnel, preventing large scale contamination of the hands when touching blood, body fluids, secretions, excretions, mucous membranes, and non-intact skin. In the United States, the Occupational Safety and Health Administration has a mandated wearing glove to reduce the risk of blood borne pathogen infections. Second, gloves are worn to reduce the likelihood that microorganisms present on the hands of personnel will be transmitted to patients during invasive or other patient-care procedures that involve touching a patient's mucous membranes and nonintact skin.

Surface sanitation:-

Sanitizing surfaces is part of nosocomial infection in health care environments. Modern sanitizing methods such as Non-flammable Alcohol Vapor in Carbon Dioxide systems have been effective against gastroenteritis, MRSA, and influenza agents. Use of hydrogen peroxide vapor has been clinically proven to reduce infection rates and risk of acquisition. Hydrogen peroxide is effective against endospore-forming bacteria, such as *Clostridium difficile*, where alcohol has been shown to be ineffective.

Antimicrobial surfaces:-

Micro-organisms are known to survive on inanimate 'touch' surfaces for extended periods of time. This can be especially troublesome in hospital environments where patients with immunodeficiencies are at enhanced risk for contracting nosocomial infections.

Touch surfaces commonly found in hospital rooms, such as bed rails, call buttons, touch plates, chairs, door handles, light switches, grab rails, intravenous poles, dispensers (alcohol gel, paper towel, soap), dressing trolleys, and counter and table tops are known to be contaminated with *Staphylococcus.*

Treatment:-

Among the categories of bacteria most known to infect patients are the category MRSA (resistant strain of *S. aureus*), member of gram-positive bacteria and *Acinetobacter* (*A. baumannii*), which is gram-negative. While antibiotic drugs to treat diseases caused by gram-positive MRSA are available, few effective drugs are available for *Acinetobacter*.*Acinetobacter* bacteria are evolving and becoming immune to existing antibiotics, so in many cases, polymyxin-type antibacterial need to be used.

Concept of Clean area

BS 5295 Clean room Standards

Custom Designed Clean room

Clean room With Custom Door Option

Custom Control Panel For Clean room Manufacture

Blower For Clean room

Typically used in manufacturing or scientific research, a clean room is a controlled environment that has a low level of pollutants such as dust, airborne microbes, aerosol particles, and chemical vapors. To be exact, a clean room has a controlled level of contamination that is specified by the number of particles per cubic meter at a specified particle size. The ambient air outside in a typical city environment contains 35,000,000 particles per cubic meter, 0.5 mm and larger in diameter, corresponding to an ISO 9 clean room which is at the lowest level of clean room standards.

Clean room Overview

Clean rooms are used in practically every industry where small particles can adversely affect the manufacturing process. They vary in size and complexity, and are used extensively in industries such as semiconductor manufacturing, pharmaceuticals, biotech, medical device and life sciences, as well as critical process manufacturing common in aerospace, optics, military and Department of Energy.

A clean room is any given contained space where provisions are made to reduce particulate contamination and control other environmental parameters such as temperature, humidity and pressure. The key component is the High Efficiency Particulate Air (HEPA) filter that is used to trap particles that are 0.3 micron and larger in size. All of the air delivered to a clean room passes through HEPA filters, and in some cases where stringent cleanliness performance is necessary, Ultra Low Particulate Air (ULPA) filters are used.

Personnel selected to work in clean rooms undergo extensive training in contamination control theory. They enter and exit the clean room through airlocks, air showers and/or gowning rooms, and they must wear special clothing designed to trap contaminants that are naturally generated by skin and the body.

Depending on the room classification or function, personnel gowning may be as limited as lab coats and hairnets, or as extensive as fully enveloped in multiple layered bunny suits with self contained breathing apparatus.

Clean room clothing is used to prevent substances from being released off the wearer's body and contaminating the environment. The clean room clothing itself must not release particles or fibers to prevent contamination of the environment by personnel. This type of personnel contamination can degrade product performance in the semiconductor and pharmaceutical industries and it can cause cross-infection between medical staff and patients in the healthcare industry for example.

Clean room garments include boots, shoes, aprons, beard covers, bouffant caps, coveralls, face masks, frocks/lab coats, gowns, glove and finger cots, hairnets, hoods, sleeves and shoe covers. The type of clean room garments used should reflect the clean room and product specifications. Low-level clean rooms may only require special shoes having completely smooth soles that do not track in dust or dirt. However, shoe bottoms must not create slipping hazards since safety always takes precedence. A clean room suit is usually required for entering a clean room. Class 10,000 clean rooms may use simple smocks, head covers, and booties. For Class 10 clean rooms, careful gown wearing procedures with a zipped cover all, boots, gloves and complete respirator enclosure are required.

Clean room Air Flow Principles

Clean rooms maintain particulate-free air through the use of either HEPA or ULPA filters employing laminar or turbulent air flow principles. Laminar, or unidirectional, air flow systems direct filtered air downward in a constant stream. Laminar air flow systems are typically employed across 100% of the ceiling to maintain constant, unidirectional flow. Laminar flow criteria is generally stated in portable work stations (LF hoods), and is mandated in ISO-1 through ISO-4 classified clean rooms.

Proper clean room design encompasses the entire air distribution system, including provisions for adequate, downstream air returns. In vertical flow rooms, this means the use of low wall air returns around the perimeter of the zone. In horizontal flow applications, it requires the use of air returns at the downstream boundary of the process. The use of ceiling mounted air returns is contradictory to proper clean room system design.

Clean room Classifications

Clean rooms are classified by how clean the air is. In Federal Standard 209 (A to D) of the USA, the number of particles equal to and greater than 0.5mm is measured in one cubic foot of air, and this count is used to classify the clean room. This metric nomenclature is also accepted in the most recent 209E version of the Standard. Federal Standard 209E is used domestically. The newer standard is TC 209 from the International Standards Organization. Both standards classify a clean room by the number of particles found in the laboratory's air. The clean room classification standards FS 209E and ISO 14644-1 require specific particle count measurements and calculations to classify the cleanliness level of a clean room or clean area. In the UK, British Standard 5295 is used to classify clean rooms.

Clean rooms are classified according to the number and size of particles permitted per volume of air. Large numbers like "class 100" or "class 1000" refer to FED_STD-209E, and denote the number of particles of size 0.5 mm or larger permitted per cubic foot of air. The standard also allows interpolation, so it is possible to describe e.g. "class 2000."

Small numbers refer to ISO 14644-1 standards, which specify the decimal logarithm of the number of particles 0.1 µm or larger permitted per cubic meter of air. So, for example, an ISO class 5 clean room has at most 105 = 100,000 particles per m^3.

Both FS 209E and ISO 14644-1 assume log-log relationships between particle size and particle concentration. For that reason, there is no such thing as zero particle concentration. Ordinary room air is approximately class 1,000,000 or ISO 9.

ISO 14644-1 Clean room Standards

Class	maximum particles/m^3						FED STD 209E equivalent
	>=0.1 µm	>=0.2 µm	>=0.3 µm	>=0.5 µm	>=1 µm	>=5 µm	
ISO 1	10	2					
ISO 2	100	24	10	4			
ISO 3	1,000	237	102	35	8		Class 1
ISO 4	10,000	2,370	1,020	352	83		Class 10
ISO 5	100,000	23,700	10,200	3,520	832	29	Class 100
ISO 6	1,000,000	237,000	102,000	35,200	8,320	293	Class 1,000
ISO 7				352,000	83,200	2,930	Class 10,000

ISO 8				3,520,000	832,000	29,300	Class 100,000
ISO 9				35,200,000	8,320,000	293,000	Room Air

Design of aseptic area:-

Asepsis is the state of being free from disease-causing contaminants (such as pathogenic bacteria, viruses, pathogenic fungi, and parasites) or, preventing contact with microorganisms. The term **asepsis** often refers to those practices used to promote or induce asepsis in an operative field in surgery or medicine to prevent infection. Ideally, a surgical field is "sterile," meaning it is free of all biological contaminants, not just those that can cause disease, putrefaction, or fermentation, but that is a situation that is difficult to attain, especially given the patient is often a source of infectious agents. Therefore, there is no current method to safely eliminate all of the patients' contaminants without causing significant tissue damage.

Aseptic technique

Aseptic technique refers to a procedure that is performed under sterile conditions. This includes medical and laboratory techniques, such as with cultures. It includes techniques like flame sterilization. The largest example of aseptic techniques is in hospital operating theatres. Aseptic technique is the effort taken to keep patients as free from hospital micro-organisms as possible (Crow 1989). It is a method used to protect wounds and other susceptible sites from organisms that could cause infection. This can be achieved by ensuring that only sterile equipment and fluids are used during invasive medical and nursing procedures. In an operating room, while all members of the surgical team should demonstrate good aseptic technique.

Method:-

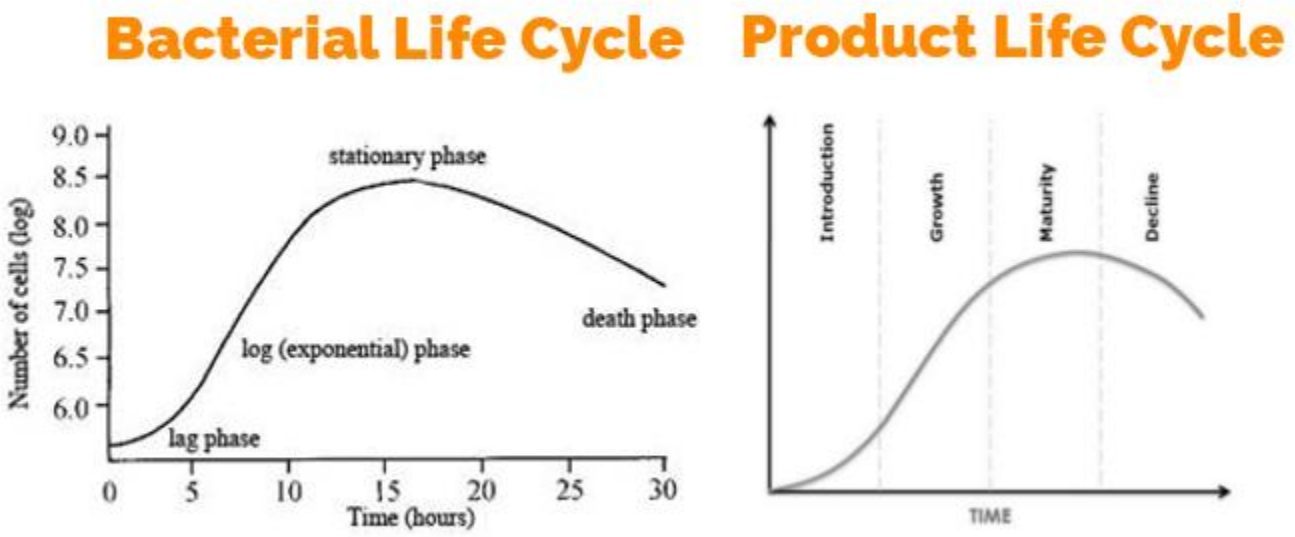
Today's techniques include a series of steps that complement each other. Foremost remains good hygienic practice. The procedure room is laid out according to specific guidelines, subject to regulations concerning filtering and airflow, and kept clean between surgical cases. A patient who is brought for the procedure is washed and wears a clean gown. The surgical site is washed, possibly shaved, and skin is exposed to a germicide (e.g., an iodine solution such as betadine). In turn, members of the surgical team wash hands and arms with germicidal solution. Operating surgeons and nurses wear sterile gowns and gloves. Hair is covered and a surgical mask is worn. Instruments are sterilized through autoclaving, or, if disposable, are used once. Irrigation is used in the surgical site. Suture material orxenografts have been sterilized beforehand. Dressing material is sterile.

Bacterial growth cycle (life cycle of bacteria)

Bacterial growth is the asexual reproduction, or cell division, of a bacterium into two daughter cells, in a process called binary fission. Providing no mutational event occurs the resulting daughter cells are genetically identical to the original cell. Hence, "local doubling" of the bacterial population occurs. Both daughter cells from the division do not necessarily survive. However, if the number surviving exceeds unity on average, the bacterial population undergoes exponential growth. The measurement of an exponential bacterial growth curve in batch culture was traditionally a part of the training of all microbiologists; the basic means requires bacterial

enumeration (cell counting) by direct and individual (microscopic, flow cytometry), direct and bulk (biomass), indirect and individual (colony counting), or indirect and bulk (most probable number, turbidity, nutrient uptake) methods. Models reconcile theory with the measurements.

Phages:-



In autecological studies, the growth of bacteria (or other microorganisms, as protozoa, microalgae or yeasts) in batch culture can be modeled with four different phases: **lag phase** (A), **log phase** or **exponential phase** (B), **stationary phase** (C), and **death phase**(D).

1. During **lag phase**, bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide. During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs.
2. The **log phase** (sometimes called the logarithmic phase or the *exponential phase*) is a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population. If growth is not limited, doubling will continue at a constant rate so both the number of cells and the rate of population increase doubles with each consecutive time period. For this type of exponential growth, plotting the natural logarithm of cell number against time produces a straight line. The slope of this line is the specific growth rate of the organism, which is a measure of the number of divisions per cell per unit time. The actual rate of this growth (i.e. the slope of the line in the figure) depends upon the growth conditions, which affect the frequency of cell division events and the probability of both daughter cells surviving. Under controlled conditions, cyanobacteria can

double their population four times a day. Exponential growth cannot continue indefinitely, however, because the medium is soon depleted of nutrients and enriched with wastes.

3. The **stationary phase** is often due to a growth-limiting factor such as the depletion of an essential nutrient, and/or the formation of an inhibitory product such as an organic acid. Stationary phase results from a situation in which growth rate and death rate are equal. The number of new cells created is limited by the growth factor and as a result the rate of cell growth matches the rate of cell death. The result is a "smooth," horizontal linear part of the curve during the stationary phase.
4. At **death phase** (decline phase), bacteria die. This could be caused by lack of nutrients, environmental temperature above or below the tolerance band for the species, or other injurious conditions.

This basic batch culture growth model draws out and emphasizes aspects of bacterial growth which may differ from the growth of macrofauna. It emphasizes clonality, asexual binary division, the short development time relative to replication itself, the seemingly low death rate, the need to move from a dormant state to a reproductive state or to condition the media, and finally, the tendency of lab adapted strains to exhaust their nutrients.

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