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REVIEW ARTICLE

STUDY ON PHARMACEUTICAL DEVELOPMENT AND QUALITY CONTROL OF ANTIBIOTIC INJECTION ADMINISTRATION BY PARENTERAL ROUTE FOR PUBLIC HEALTH CARE.

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Abstract

Objective of this review article to give detail about antibiotic used as parenteral route of administration in injection forms. Antibiotics history began with the observations made by Sanderson and Roberts on the inhibition of bacterial growth by other organisms, at the end of the XIX Century. Biomedical research in this field advanced importantly during World War II, after the discovery of penicillin by Fleming. Now-a-days the number of antibiotics plays an important role in the society particularly animal and human life. A series of different antibodies were quickly discovered after penicillin came into use. In 1940, Selman Waksman began searching for antibiotic compounds produced by soil microorganisms. In 1943, one of Waksman's student discovered streptomycin, leading to a flood of researchers combing that world for new drugs. It was in this same period that Rene Dubos discovered gramicidin, the first antibiotic active against gram-positive bacteria. Many discoveries were of drugs that were too toxic for human use, or that had already been discovered. Injection as antibiotic are administration by parenteral route. It gave more accurate and efficient on set of action to the patients.

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Introduction:-

In the last 60 years, major improvements in the initial recognition and the treatment of infectious diseases have resulted in an extraordinary reduction in the morbidity and mortality associated with these illnesses. This has been due, in part, to our better sympathetic of the fine molecular biological mechanisms of these diseases and to our improved understanding of their pathophysiology and their epidemiology but, most notably, to the rapid improvement of safe and effective new antimicrobial treatments that have been able to round the specific agent causing the infection, thus helping the infected host to eliminate the infection being treated.

Antibiotics:-

An antibiotic is an agent that inhibit or kill the growth of microorganism. ^[1] The term antibiotic was first used in 1942 by American microbiologist and biochemist Selman Abraham Waksman and his collaborators in Journal

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articles to describe any substance produced by microorganism that is antagonistic to the growth of other microorganism in high dilution.^[2]

History of Antibiotic development:-

It was in the early 1900's when Paul Ehrlich first hypothesized that dyes could be used as antimicrobial drugs, based on their differential affinities for various tissues. In 1904, Ehrlich and Shiga discovered that a red dye called trypanrot was effective against trypanosomes.^[3] It was around this time that arsenicals drew Ehrlich's interest. Ehrlich, along with Sahachiro Hata in 1909, found that arsphenamine (named Salvarsan) was active against spirochetes and, therefore, was an effective cure for syphilis. The first truly effective class of antimicrobial drugs were the sulfonamides, discovered by Gerhard Domagk. In 1932, two scientists at the Bayer company, Mietzsch and Klarer, synthesized Prontosil red, a red dye bound to a sulfonamide group. Domagk showed, in 1935, that infections in mice caused by hemolytic *Streptococci* were cured by Prontosilred.^{[4][5]} Unfortunately for Bayer, Prontosil red was shown to have no antibacterial activity in vitro. Although Penicillin was the first natural antibiotic to be discovered, the idea of using microorganisms therapeutically was not new. Fungi had been used in poultices for many years, and by 1899 a product called pyocyanase, which was an extract from *Pseudomonas aeruginosa*, was used in the treatment of wounds. Penicillin was first isolated from *Penicillium notatum* in 1928 by Alexander Fleming,^[6] but he was unable to isolate and purify enough drug to be of any use. By 1941, Ernst Chain, Howard Florey and Norman Heatley had shown the therapeutic value of penicillin, but they were also unable to produce enough penicillin for commercial use. Collaboration with Andrew Moyer and Robert Coghill at the US FDA's Northern Regional Research Laboratory in Illinois led to much higher production yields of penicillin by 1943.^{[7][8]} After a worldwide search for *Penicillium* strains that could produce more penicillin, Raper and Fennel found a strain of *Penicillium chrysogenum* on a moldy cantaloupe at a local market that was capable of even higher yields of penicillin.^{[9][10]}

For example, some antibiotic producing bacteria were isolated from a wound infection and others from a sewage, a chicken's throat, and a wet patch of wall in Paris. In 1962, one of the later discoveries was a synthetic drug, nalidixic acid, the first of the quinolones to be described, and although not therapeutically important by itself, modification of nalidixic acid led to the production of the highly effective fluoroquinolones. Members of this class, such as ciprofloxacin, norfloxacin, enrofloxacin and ofloxacin have become very important in the treatment of the diseases in both humans and animals.^[11]

Classification of Antibiotics:-

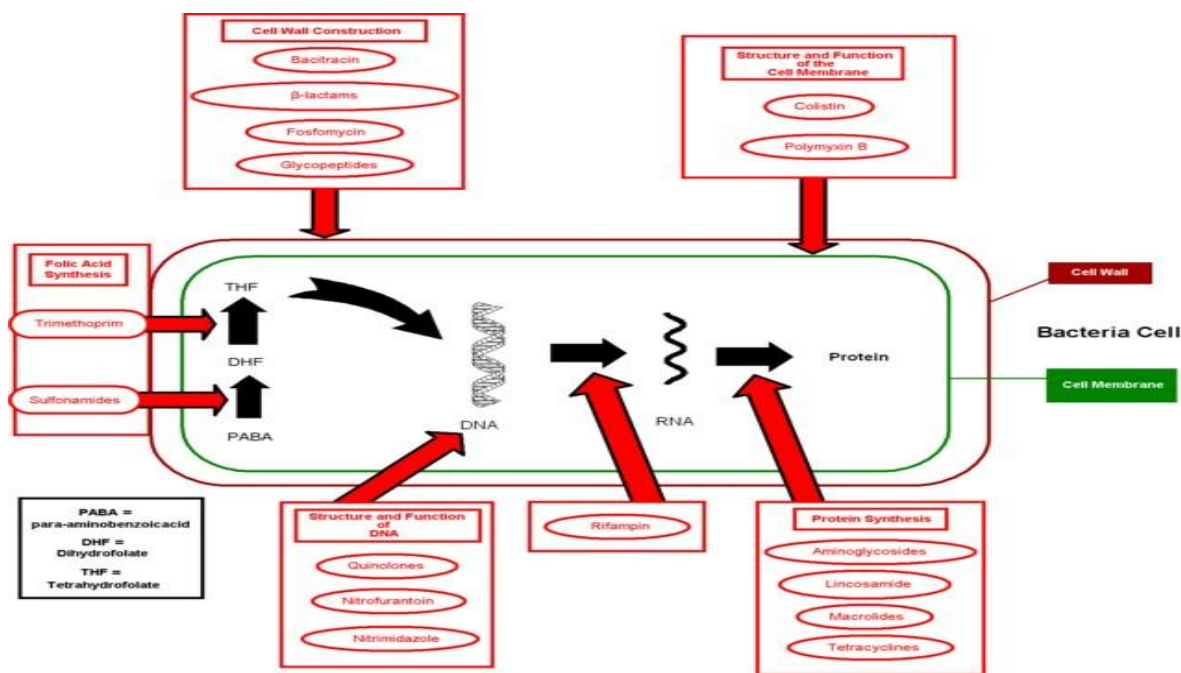
Antimicrobial drugs can be classified according to their chemical structure and mechanism of action.^[12]

Table 1.1:- Classification: Chemical structure.

Class	Examples
Sulfonamides and related drugs	sulfadiazine; dapsone; paraminosalicylic acid
Quinolones	nalidixic acid; norfloxacin; ciprofloxacin
Beta lactam	penicillin; cephalosporin; carbapenem
Tetracycline	oxytetracycline; doxycycline
Aminoglycosides	streptomycin; gentamycin; amikacin
Macrolide	erythromycin; azithromycin
Glycopeptide	vancomycin; teicoplanin
Nitroimidazole	metronidazole; ornidazole
Azole derivatives	miconazole; clotrimazole
Oxazolidinone	Linezolid

Table 1.2:- Classification: Mechanism of action.

Mechanisms of Action	Examples
Inhibit cell wall synthesis	penicillin; cephalosporin; vancomycin
Cause leakage from cell membrane	colistin; nystatin
Inhibit protein synthesis	tetracycline; chloramphenicol; linezolid
Cause misreading of Mrna	streptomycin; gentamycin;
Inhibit DNA gyrase	Ciprofloxacin
Interfere with DNA function	rifampin; metronidazole; ornidazole
Interfere with DNA synthesis	acyclovir; zidovudine
Interfere with intermediary metabolism	sulfonamides; trimethoprim

Figure 1.1:- Mechanism of action of Antimicrobial agent^[13]**Parenterals:-**

Parenteral (Greek, *para – beside and enteron - intestine*) formulations are injected directly into body tissue through the primary protective systems of the human body, the skin, and mucous membranes. Parenteral preparations are sterile preparations intended for administration by injection, infusion or implantation into the human or animal body.^[22] Certain pharmaceutical agents, particularly peptides, proteins, and many chemotherapeutic agents, can only be given parenterally, because they are inactivated in the gastrointestinal tract when given by mouth.

Parenteral preparations may require the use of excipients, for example to make the preparation isotonic with blood, to adjust the pH, to increase solubility, to prevent deterioration of the active substances or to provide adequate antimicrobial properties but not to adversely affect the intended medicinal action of the preparation or, at the concentrations used, to cause toxicity or undue local irritation. Parenteral preparations must be free from visible particulate matter and also from pyrogenic (endotoxin) contamination. Parenteral preparation must be compatible, if applicable, with IV diluents, delivery systems and other drug products co-administered. Most of the parenteral preparation are of immediate action, which is in the form of solution, suspension or emulsion.^[23]

Routes of administration:-^[24]

1. **Intra-cutaneous or Intra-dermal:** These are made into the skin, between the inner layer, or dermis, and the outer layer, or epidermis.
2. **Subcutaneous or Hypodermic:** These are made under the skin, into the subcutaneous tissue.
3. **Intramuscular:** These are made into a muscle, the needle passing through the skin, subcutaneous tissue and the membrane enclosing the muscle, and opening into muscle tissue.
4. **Intra-venous:** These are made into vein and, therefore, are introduced directly into the blood stream.
5. **Intra-arterial:** These are given directly into the artery when an immediate effect in a peripheral area is required.
6. **Intra-cardiac:** These are given into the heart muscle or ventricle in an emergency only.
7. **Intra-thecal:** These are made into the subarachnoid space that surrounds the spinal cord.
8. **Viii. Intra-cisternal :** These are inserted in the mid- line between the atlas and axis (first and second cervical vertebrae respectively) and directed forward and upwards.
9. **Peridural :** These are given between the duramatter and the inner aspects of the vertebrae.
10. **Intra-articular:** These are made into the synovial fluid the liquid that lubricates the articulating ends of bones in a joint.

Intravenous Subcutaneous

Intra-dermal

Intramuscular.



Epidermis

Dermis

Sub

Subcutaneous adipose tissue

Muscle

Figure 1.3:- Routes of Parenteral administration ^[25]**Table 1.3:-**Parenteral Routes ^[26]

Route	Injection site
Intravenous (IV)	Vein
Intramuscular (IM)	Muscle tissue
Intradermal (ID)	Dermis of the skin
Subcutaneous (SC)	Subcutaneous tissue of the skin
Intrathecal (IT)	Sub arachnoid space of the spinal cord
Epidural	Epidural space of the spinal cord
Intra-arterial	Artery
Intra-articular	Joint space
Intracardiac	Heart
Intra ocular	Eye
Intra peritoneal	Peritoneal cavity

Types of Parenteral preparations: It can be categorized as ^[27]

- Small scale dispensing – Usually one unit at a time.
- Large scale manufacturing – In this hundreds of thousands may constitute one lot of product.

Table 1.4:-Difference between Large volume parenteral and Small volume parenteral.

Parameter	Large volume parenteral	Small volume parenteral
Volume	101 – 1000 ml	100 ml or less
Route	IV	IV, IM, SC
Dose unit	Single	Single or multiple
Needle	1 ½, 18-19 gauze	1 ½, 20-22 gauze
Preservative	Not used	Used
Buffer	Not used	Used
Formulation	Solution and o/w nutrient emulsion	Solution, emulsion, suspension
Use	As nutrition in detoxification Aid during surgery	As therapeutic As diagnostic agents

Several categories of parenteral preparation may be distinguished :^[28]

- injections
- infusions
- concentrates for injections or infusions
- powders for injections or infusions
- implants

Injections: Injections are sterile solutions, emulsions or suspensions. They are prepared by dissolving, emulsifying or suspending the active substance(s) and any added excipients in Water for injections, in a suitable, sterile non-aqueous liquid or in a mixture of these vehicles.

Infusions: Infusions are sterile solutions, aqueous solutions or emulsions with water as a continuous phase; they are usually made isotonic with blood. They are principally intended for administration in large volume. Infusion do not contain any added antimicrobial preservative.

Concentrates for injections or infusions: Concentrates for injections or infusions are **sterile** solutions intended for injection or infusion after dilution. They are diluted to a prescribed volume with a prescribed liquid before administration. After dilution, they comply with the requirements for injections or for infusions.

Powders for injections or infusions: Powder for injections or infusions are solid, sterile substances distributed in their final containers and which, when shaken with the prescribed volume of a prescribed sterile liquid, rapidly form either clear and practically particle-free solutions or uniform suspensions. After dissolution or suspension, they comply with the requirements for injections or for infusions.

Implants: Implants are sterile solid preparations of a size and shape suitable for parenteral implantation and release the active substance(s) over an extended period of time. Each dose is provided in a sterile container.

Formulation of Parenterals:^[29]

- 1 Active drug
- 2 Added substances
 - Antimicrobial agent
 - Buffer
 - Antioxidant
 - Tonicity agent
 - Chelating agent
 - Complexing and surface active agent
 - Solubilizers
3. Vehicle - Aqueous - Non-aqueous

Active drug:-

It is active pharmaceutical ingredient. A thorough evaluation of properties of the active drug or drug is essential in developing a stable and safe parenteral dosage form.

Added substances:-

Antimicrobial agent:-

Substance that kill or slow the growth of microbes. Antimicrobial agent serves to maintain the sterility of the product during its shelf life and use. They are required in preparations intended for multiple dosing from the same container because of the finite probability of accidental contamination during repeated use. They are also included, in some single dose products to provide additional assurance of product sterility. Most commonly used parenteral antimicrobial preservative includes phenylmercuric nitrate and thiomersol 0.01%, benzethonium chloride and benzalkonium chloride, phenol or cresol 0.5%, chlorobutanol 0.5%, methyl paraben, propyl paraben.^{[30][31]}

Buffer:-

Buffers are added to a formulation to adjust the pH in order to optimize solubility and stability. For parenteral preparations, the pH of the product should be close to physiologic pH. The selection of buffer concentration (ionic strength) and buffer species are important. Citrate and acetate buffer, phosphate buffer.^[32]

Antioxidant:-

Salts of sulfur dioxide, including bi sulfite, metabisulfite, and sulfite are the most common antioxidants used in aqueous parenterals. These antioxidants maintain product stability by being preferentially oxidized and gradually consumed over shelf life of the product. Irrespective of which salts is added to the solution, the antioxidant moiety depends on the final concentration of the compound and the final pH of the formulation.^[33]

Tonicity agent:-

It is important that injectable solutions that are to be given intravenously are isotonic, or nearly so. Because of osmotic pressure changes and the resultant exchange of ionic species across red blood cell membranes, non isotonic solutions, particularly if given in quantities larger than 100 ml, can cause haemolysis or crenation of red blood cells. Tonicity can be measured by osmometer and fragility point. Electrolytes : sodium chloride; Nonelectrolytes : glucose, mannitol, glycerine; Isotonic : dextrose injection 5% and sodium chloride injection 0.9%.^[34]

Chelating agent:-

Only a limited number of chelating agents are used in parenteral products. They serve to complex heavy metals and therefore can improve the efficacy of antioxidants or preservatives. disodium edta, citric acid, tartaric acid and some amino acids also can act as chelating agents.^[35]

Complexing and surface active agent:-

Increase and maintain drug solubility. Examples include complexing agents and surface active agents. The most commonly used complexing agents are the cyclodextrins, including captisol. The most commonly used surface active agents are polyoxyethylenesorbitanmonolaurate (tween 20) and polyoxyethylenesorbitanmonooleate (tween 80).^[36]

Solublizers:-

Solublizers are used to enhance and maintain the aqueous solubility of poorly water soluble drugs.^[37]

Vehicles:-

Aqueous: It can be water and water miscible liquids.

Water: Most liquid injections are quite dilute, the component present in the highest proportion is the vehicle. The vehicle of greatest importance for parenteral products is water. WFI is highly purified water used as a vehicle for injectable preparations which will be subsequently sterilized. USP requirements include not more than 10 parts per million of total solid. pH of 5 - 7 WFI may be prepared by either distillation or reverse osmosis. Stored for less than 24 hr at RT or for longer times at specific temperature should meet USP pyrogen test.^[38]

Water miscible: A number of solvents that are miscible with water have been used as a portion of the vehicle in the formulation of parenteral. These solvents are used to solubilize certain drugs in an aqueous vehicle and to reduce hydrolysis.

The most important solvents in this group are ethyl alcohol, liquid polyethylene glycol, and propylene glycol.

Ethyl alcohol is used in the preparation of solutions of cardiac glycosides and the glycols in solutions of barbiturates, certain alkaloids, and certain antibiotics. Such preparations are given intramuscularly.

There are limitations with the amount of these co-solvents that can be administered, due to toxicity concerns, greater potential for haemolysis, and potential for drug precipitation at the site of injection.^[39]

Non-Aqueous : The most important group of non-aqueous vehicles is the fixed oils. The oils most commonly used are corn oil, cottonseed oil, peanut oil, and sesame oil. Fixed oils are used as vehicles for certain hormone (eg., progesterone, testosterone, deoxycorticosterone) and vitamin (eg., Vitamin K, Vitamin E) preparations.^[40]

Table 1.5:-Advantages and Disadvantages of Parenteral Products ^[41]

Advantages		Disadvantages	
<input type="checkbox"/>	Provides drug and nutritional options for patients unable to tolerate oral therapy	<input type="checkbox"/>	Difficulty/ impossibility of drug removal/ reversal
<input type="checkbox"/>	Circumvents absorption limitations of gastrointestinal tract	<input type="checkbox"/>	Risk of infection
<input type="checkbox"/>	Quick onset of action	<input type="checkbox"/>	Risk of emboli
<input type="checkbox"/>	Localized delivery	<input type="checkbox"/>	Risk of hypersensitivity reactions
<input type="checkbox"/>	Prolonged duration of effect	<input type="checkbox"/>	Higher costs

General Guidance for Developing Formulations of Parenteral Drugs:-

The final formulation of a parenteral drug product depends on understanding the following factors that dictate the choice of formulation and dosage form.

1. **Route of administration** — Injections may be administered by such routes as intravenous, subcutaneous, intradermal, intramuscular, intra articular, and intra thecal. The type of dosage form (solution, suspension, etc.) determines the particular route of administration employed.
2. If the **route of administration** must be intravenous, then only solutions or micro-emulsions can be the dosage form.
3. **Pharmacokinetics of the drug** — **Rates of absorption** (for routes of administration other than intravenous or intra-arterial), **distribution, metabolism, and excretion** for a drug have some effect on the selected route of administration and, accordingly, the type of formulation.
4. **Drug solubility** — If the drug is insufficiently soluble in water at the required dosage, then the formulation must contain a co-solvent or a solute that sufficiently increases and maintains the drug in solution. If relatively simple formulation additives do not result in a solution, then a dispersed system dosage form must be developed.
5. **Drug stability** — If the drug has significant degradation problems in solution, then a freeze-dried or other sterile solid dosage form must be developed. Stability is sometimes affected by drug concentration that, in turn, might affect size and type of packaging system used.
6. **Compatibility of drug** with potential formulation additives and packaging systems — It is wellknown that drug-excipient incompatibilities frequently exist. Initial preformulation screening studies are essential to ensure that formulation additives, although possibly solving one problem, will not create another.
7. The **use of silicone** to lubricate vial rubber closures, syringe rubber plungers to coat the inner surface of glass syringes, and cartridges potentially can induce protein aggregation.
8. **Desired type of packaging** — Selection of packaging (i.e. type, size, shape, color of rubber closure, label, and aluminum cap) is often based on marketing preferences and competition.^[42]

Main Steps Involved in the Formulation of a New Parenteral Drug Product:

Physical properties of active drug substance - Structure, molecular weight

- Solubility in water at room temperature
- Effect of pH on solubility
- Solubility in certain other solvents
- Unusual solubility properties
- Isoelectric point for a protein or peptide
- Hygroscopicity
- Potential for water or other solvent loss
- Aggregation potential for protein or peptide

Chemical properties of active drug substance

- 'Validated' analytical method for potency and purity
- 10% degradation at room temperature in aqueous solution in the pH range of anticipated use
- Time for 10% degradation at 5°C
- pH stability profile
- Sensitivity to oxygen

- Sensitivity to light
- Major routes of degradation and degradation products

Initial formulation approaches

- Single dose vs multiple dose
- Shelf life goals
- High temperature storage
- Temperature cycling
- Light and/or oxygen exposure
- Selection of primary container and closure ^[43]

Materials:-**Water for Injection:-**

It can be prepared by distillation or by membrane technologies (i.e., reverse osmosis or ultra filtration). The water must be pretreated by one or a combination of the following treatments: chemical softening, filtration, de ionization, carbon adsorption, or reverse osmosis purification. Distillation is a process of converting water from a liquid to its gaseous form (steam). Since steam is pure gaseous water, all other contaminants in the feed water are removed. ^[44]

Glass:-

Glass is employed as the container material of choice for most SVIs. It is composed, principally, of silicon dioxide, with varying amounts of other oxides, such as sodium, potassium, calcium, magnesium, aluminum, boron, and iron. ^[45]

Types:-

The USP provides a classification of glass:

- Type I, a borosilicate glass;
- Type II, a soda-lime treated glass;
- Type III, a soda-lime glass; and
- NP, a soda-lime glass not suitable for containers for parenteral.

Type I glass will be suitable for all products, although sulfur dioxide treatment is sometimes used for even greater resistance to glass leach-ables. Because cost must be considered, one of the other, less expensive types may be acceptable.

Type II glass may be suitable, for example, for a solution that is buffered, has a pH below 7, or is not reactive with the glass.

Type III glass is usually suitable for anhydrous liquids or dry substances.

Types II and III glass compounds are composed of relatively high proportions of sodium oxide (~14%) and calcium oxide (~8%). This makes the glass chemically less resistant. Both types melt at a lower temperature, are easier to mold into various shapes.

Type II glass has a lower concentration of the migratory oxides than Type III. In addition, Type II has been treated under controlled temperature and humidity conditions, with sulfur dioxide or other de alkalizers to neutralize the interior surface of the container.

The glass types are determined from the results of two USP tests:

The Powdered Glass Test

The Water Attack Test.

The Powdered Glass Test challenges the leaching potential of the interior structure of the glass, whereas the Water Attack Test challenges only the intact surface of the container.

Selecting the appropriate glass composition is a critical facet of determining the overall specifications for each parenteral formulation. Glass can be the source / cause of leach-ables / extractable, particulates (glass deamination or glass lamellae formation), adsorption of formulation components, especially proteins, and cracks / scratches.

Plastic:-

Plastic packaging has always been important for ophthalmic drug dosage forms and is gaining in popularity for injectable dosage forms. Plastic bottles for large volume injectable (LVIs) have been used for many years. Plastic vials for SVIs may be a wave of the future plastic packing offers such advantages of cost savings elimination of the problems caused by breakage of glass and increase convenience of use.

[46]

Rubber:-

Rubber formulations are used as rubber closures, rubber plungers and other applications. The most common rubber polymers used in SVIs closures are natural and butyl rubber. Silicone and neoprene also are used but less frequently in sterile products. Butyl rubber has great advantages over natural rubber in that butyl rubber requires fewer additives, has low water vapor permeation properties and has good characteristics with respect to gaseous permeation reactivity with the active ingredient.^[47]

Labeling:-

The package and in particular, the labeling for parenteral dosage forms are integral and critical parts of the product. The labeling must be legible and clearly identify the drug, its concentration, handling or storage conditions and any special precautions, the dose or concentration must be predominantly displayed when other concentrations of the same drug are marketed, proper labeling is difficult with the space limitation dictated by small containers used for many parenteral products. Smaller containers have become increasingly popular because of the unit dose concept.^[48]

Controlled environment required for parenteral preparation:-

Clean Room Classified Areas: Due to the extremely high standards of cleanliness and purity that must be met by parenteral products, it has become standard practice to prescribe specifications for the environments (clean rooms) in which these products are manufactured.^[49]

The Critical and General area of clean room:

The clean room divides into

1. Critical Area
2. General Area

The critical area is the area around the point of the production where contamination can gain direct access to the process. This area often protected by localized laminar flow clean benches and workstations. The General area is the rest of the clean room where contamination will not gain direct entry into the product but should be kept clean because of the transfer of contamination into the critical area. It is necessary that the critical area be cleaned most often with the best cleaning ability without introducing contamination. [50][51][52]

Classification of Clean Rooms:-

The class is directly related to the number of particles per cubic foot of air equal to or greater than 0.5 micron.

1. Class 100,000: Particle count not to exceed a total of 100,000 particles per cubic foot of a size 0.5 μ and larger or 700 particles per foot of size 5.0 μ and larger.
2. Class 10,000: Particle count not to exceed a total or 10,000 particles per cubic foot of a size 0.5 μ and larger or 65-70 particles per cubic foot of a size 5.0 μ and larger.
3. Class 1,000: Particles count not to exceed a total of 1000 particles per cubic foot of a size 0.5 μ and larger or 10 particles per cubic foot of a size 5.0 μ and larger.
4. Class 100: Particles count not to exceed a total of 100 particles per cubic foot of a size 0.5 μ and larger.[53][54][55]

Class 1: The particle count shall not exceed a total of 3000 particles/m³ of a size 0.5 μ .

Class 2: The particle count shall not exceed a total of 3000 particles/m³ of a size of 0.5 μ or greater; 2000 particles/m³ of size 0.5 μ or greater; 30 particles of a size 10 μ .

Class 3: The particle count shall not exceed a total of 1,000,000 particles of a size of 1μ or greater; 20,000 particles/ m^3 of size 5μ or greater; 4000 particles/ m^3 of a size 10μ or greater; 300 particles of a size of 25μ or greater.
 Class 4: The particle count shall not exceed a total of 200,000 particles of a size of 5μ or greater^{[54][55]} For the manufacture of sterile medicinal products normally 4 grades can be distinguished:

GRADE "A": The local zone for high risk operations. eg. filling zone, stopper bowls, open ampules and vials.

GRADE "B": In case of aseptic preparation and filling, the back ground environment for grade "A" zone.

GRADE "C" & "D": Clean areas for carrying out less critical stages in the manufacture of sterile produce^{[54][56]}

Table 1.6:-Air borne particulate classification for Grade A, B, C and D ^{[53][54]}

Grade	Maximum permitted number of particles/ m^3			
	At rest		In n operation	
	0.5mm	5mm	0.5mm	5mm
A	3500	0	3500	0
B	3500	0	350000	2000
C	350000	2000	3500000	20000
D	3500000	20000	Not defined	Not defined

Table 1.7:-Clean room classification ^[54]

FS209 Clean room Classification	ISO 14644-1 Clean room Classification	NMT $0.5\mu m$ particles/ m^3	Viable Microbes (cfu/ m^3)	Average Airflow Velocity (fpm)	Air change/hr
100000	8	3520000	100	5 to 10	5 to 48
10000	7	35200	10	10 to 15	60 to 90
1000	6	35200	7	25 to 40	150 to 240
100	5	3520	1	40 to 80	240 to 480

Air flow velocities of 0.36 m/s to 0.56 m/s (70 fpm to 110 fpm) are recommended as standard design for laminar air flow clean room systems. Air is supplied at a much higher pressure than its surrounding area.

Liquids:-

There are three main methods for filling liquids into containers with high accuracy:

- volumetric filling, time
- pressure dosing,
- net weight filling. ^[55]

Sterilization:-

The processes (thermal and chemical) are designed to destroy or eliminate micro-biologic contaminants present in a product.

Thermal methods:-

- Most common, cost-effective and rapid means of sterilization
- Lethal effectiveness of heat on microorganisms depends upon the degree of heat, the exposure period, and the moisture present.
- The range of sterilizing temperatures, the time required to produce a lethal effect is inversely proportional to the temperature employed.
- Sterilization by thermal methods may be effected at lower temperatures in the presence of moisture. ^[56]

Thermal methods of sterilization may be divided into:

1. By dry heat
2. By moist heat
3. Radiation
4. Filtration
5. Physical cleaning

Table 1.8:-Methods of Thermal Sterilization ^[57]

Method	Recommended use	Limitation
Moist heat		
Autoclave	Sterilizing instruments, linens, utensils, treatment trays, media and other liquids	Ineffective against organisms in materials impervious to steam, cannot be used for heat sensitive articles
Free flowing steam or boiling water	Sterilizing instruments, linens, utensils, treatment trays.	Cannot be guaranteed to produce sterilization on one exposure
Dry heat		
Hot-air oven	Destruction of non spore forming pathogens, sanitizes bedding,	Destructive to materials which cannot withstand high
Incineration	clothing, and dishes	temperatures for long duration
Radiation		
Ultraviolet rays	Disposal of contaminated objects that cannot be reused.	Size of incinerator must be adequate to burn largest load; potential of air pollution
X-ray, gamma, and cathode radiation	Control of air borne infection; disinfection of surfaces	Must be absorbed to be effective (does not pass through transparent glass or opaque objects); irritating to eyes and skin; low penetration expensive and requires special facilities for use
Filtration		
Membrane filters	Sterilization of heat- sensitive surgical materials and other medical devices	Fluids must be relatively free of particulate matter Expensive
Fiber glass filters (HEPA)	Sterilization of heat- sensitive surgical materials and other medical devices	Fluids must be relatively free of particulate matter Expensive
Physical cleaning		
Ultrasonics	Sterilization of heat- sensitive biological fluids	Not effective alone, but as adjunct procedure enhances effectiveness of other methods
Washing	Air disinfection Effective in decontaminating delicate cleaning instruments hands, skin, objects	Sanitizes; reduces microbial flora

Chemical methods:- ^[58]

Chemical methods are used for sterilization. Although heating provides the most reliable way to rid objects of all transmissible agents, it is not always appropriate, because it will damage heat sensitive materials such as biological materials, fiber optics, electronics and many plastics. Low temperature gas sterilizers function by exposing the articles to be sterilized to high concentrations (typically 5 to 10% v/v) of very reactive gases (alkylating agents such as ethylene oxide, and oxidizing agents such as hydrogen dioxide and ozone)

1. Ethylene oxide: (EO or EtO) Commonly used to sterilize the the objects that are sensitive to temperature greater than 60°C. EO treatment is generally carried out between 30°C and 60°C with relative humidity above 30% and gas concentration between 200 and 800 mg/l and typically lasts for at least 3 hours.
2. Nitrogen dioxide: (NO₂) Used against wide range of microorganisms, including common bacteria, viruses and spores. The mechanism for lethality is the degradation of DNA in the spore core through nitration of the phosphate backbone, which kills the exposed organism as it absorbs (NO₂).
3. Ozone: Used in industrial setting to sterilize water and air. It has benefit of being able to oxidize most organic matter.
4. Hydrogen peroxide: It is a strong oxidant and these oxidizing properties allow it to destroy a wide range of pathogens and is used to sterilize heat or temperature sensitive articles.

Applications of sterilization:-

1. Accelerated electrons or gamma rays may be used to sterilize select products by a continuous process.
2. Vitamins, antibiotics and hormones in dry state have been successfully sterilized by radiation.

Evaluation Parameters:-^[59]

The basic quality control test which are performed on sterile parenteral product includes:

- Sterility test
- Pyrogen test
- Leaker test
- Particulate matter test
- Uniformity of mass
- Bacterial Endotoxin Test

Sterility Test:- Sterility means complete absence of all viable micro-organisms. The methods which are used to perform sterility test are:

- a. Direct transfer method
- b. Membrane filtration method
- c. Direct transfer method: It is a traditional sterility test method which involves a direct inoculation of required volume of a sample in two tests containing a culture medium that is FTM, SCDM. This method is simply in theory but difficult in practice when the demand for repetition in opening container, sampling transferring, and mixing increases causes potential error in operator technique.
- d. Membrane Filtration Technique: It is official in USP 1970. This method basically involves filtration of sample through membrane filters of porosity 0.22 micron and diameter 47mm. The filtration is associated under vacuum, after filtration completion the membrane is cut into 2 halves and one half is placed in two test tubes containing FTM, SCDM medium.

Interpretation: If no visible evidence of microbial growth in culture medium in test tube then it is interpreted that the sample representing lot is without intrinsic contamination.

1. **Pyrogen Test:** Pyrogens are product of metabolism in microorganism gm-ve bacteria produces most potent pyrogen. When these pyrogens are introduced into the body they produce a mark response of fever with body ache and vasoconstriction within an onset of one hour. Pyrogen test is done on rabbit ear vein.
2. **Leaker Test:** The leaker test is intended to detect incompletely sealed ampoules so that they may be discarded. Ampoules are dipped in 1% methylene blue solution. It is performed in vacuum chamber. Vials & bottles not subjected to this test because of flexibility of rubber.
3. **Particulate matter:** Particulate matter is a primary concern in the parenteral products given by iv routes, all parenteral products should be free from insoluble particles. White screen for the detection of black particles and black screen for the detection of white particles to detect heavy particles.
4. **Uniformity of mass:** Weight variation requirements are applied to sterile solids with or without inactive substances that have been prepared from true solution and freeze-dried in the final containers. Content uniformity requirements are applied to all cases of sterile solids that contain inactive or active substances except for special products.
5. **Bacterial Endotoxin test :** The bacterial endotoxin test (BET) is a test to detect or quantify endotoxins from gram negative bacteria using *Amoebocyte lysate* from the horse shoe crab (*Limulus polyphemus* or *Tachypleustridentatus*)

Procedure:-**Preparation of Endotoxin free equipment:**

All the materials coming in direct contact with samples or test material must be free of endotoxin contamination

Glasswares shall be cleaned with purified water, rinsed with water for injection and are made endotoxin free by dry heat sterilization at 200°C for at least three hours

Reagents:-**Limulus Amoebocyte Lysate (LAL) working solution:-**

Collect LAL powder into the bottom of the vial by tapping it on hard surface. ii). Aseptically reconstitute the lyophilized LAL with endotoxin free water as indicated on the vial label. iii). Swirl gently to dissolve, avoiding liquid contact with stopper. iv). Do not invert or shake vigorously.

It is preferable to use entire solution the same day of reconstitution, although the LAL working solution may be stored frozen at 20°C upto 28 days vi). LAL can be frozen and thawed only once. vii). Re hydrated LAL should be stored on a cold surface or in refrigerator at 2- 8 during intermittent use for upto 24 hrs. viii). Lysate working solutions which forms gel or become turbid indicate contamination of reagent and should not be used.

Endotoxin standard:-

1. Reconstitute the lyophilized control standard endotoxin (CSE) with volume of endotoxin free water as indicated on the certificate of analysis (COA).
2. Vortex intermittently for 5 minutes and use this concentrate to dilutions described under endotoxin standard dilutions.
3. Preserve the concentrate in a refrigerator for making subsequent dilutions for not more than 28 days at 2–8 °C.
4. Mix vigorously, using a vortex mixer for not less than 30 seconds before proceeding to make the next dilutions.
5. Do not store dilutions, because of loss of activity by adsorption.
6. Any endotoxin solution standing for more than 30 minutes should be re-vortex for at least seconds prior to use. Diluted endotoxin should be made daily.

Preparation of sample (pH adjustment):-

1. The pH of the solution to be tested must be between 6 to 8.
2. pH may be adjusted if required with endotoxin - free 0.1 M Hydrochloric acid, or endotoxin - free 0.1M Sodium hydroxide.

Preparation of endotoxin free 0.1M NaOH (approx.) solution:-

1. Weigh about 0.1g Sodium hydroxide and add into 25 ml endotoxin free water contained in an endotoxin free bottle.
2. Stopper the bottle with rubber stopper, seal and autoclave at 121°C for 30 minutes.

Preparation of endotoxin free 0.1 M HCl (approx.) :

1. Using an endotoxin free pipette, add 100 µl of concentrated Hydrochloric acid (12 M) into 10 ml of endotoxin free water contained in an endotoxin free vial.
2. Plug the vial with rubber stopper, seal with aluminum seal and autoclave at 121°C for 20 minutes.

Precautions:-

1. pH electrodes must not be used as it may contaminate the test solution.
2. The pH of sample can be checked by applying drop of solution to pH indicator paper with endotoxin free pipettes.

Endotoxin standard dilutions:-

1. Mix endotoxin standard stock solutions for 5-10 minutes using vortex mixer.
2. All endotoxin dilutions should be prepared in sterile, endotoxin free polystyrene or glass tubes.
3. Prepare dilutions of endotoxin standard stock solution using endotoxin free water to get dilutions ranging from 4λ to $1/4\lambda$. (where λ = labeled sensitivity of LAL Reagent)
4. Test for confirmation of labeled LAL reagent sensitivity :

Confirm the labeled sensitivity of each lot of LAL reagent prior to use in the test as described below:-

Prepare a series of two fold dilutions of the CSE to give concentrations of 2λ , λ , 0.5λ and 0.25λ , where, λ is the labeled sensitivity of the LAL reagent in endotoxin unit (EU) per ml.

Conclusion:-

The objective of this review article what is antibiotic injection with comparable clinical efficacy to other antibacterial formulation of injection. In this article we gave knowledge about antibiotic form and all the details

about the formulation of injection. Its comparable clinical efficacy of their antibacterial and favorable route of administration and the pharmacokinetic and preformulation parameters for injection and microbiological studies of antibiotic injection. It is one area where major development changes are required to give more efficient effect injection and reduce side effect of it.

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