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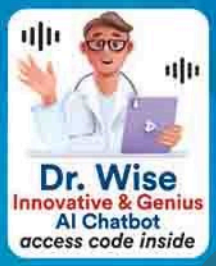

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CHAPTER 6

Bacterial Genetics

CHAPTER PREVIEW

- Principles of Bacterial Genetics
  - Bacterial DNA
  - Bacterial RNA
  - Polypeptide Synthesis
  - Plasmid
- Gene Transfer in Bacteria
  - Mutation
- Horizontal Gene Transfer
  - Transformation
  - Transduction
  - Lysogenic Conversion
  - Conjugation
  - Transposition
- Gene Transfer by Artificial Methods
  - Genetic Engineering
  - Blotting Techniques

PRINCIPLES OF BACTERIAL GENETICS

Bacterial genetics deals with the study of heredity and gene variations seen in bacteria. All hereditary characteristics of the bacteria are encoded in their DNA (deoxyribonucleic acid). Bacterial DNA is present in the chromosome as well as in extrachromosomal genetic material as plasmid.

BACTERIAL DNA

Bacteria possess a single haploid chromosome, comprising supercoiled circular double-stranded DNA of 1 mm in length. The bacterial DNA lacks basic proteins. However, some bacteria have a linear chromosomal DNA and some have two chromosomes (e.g. *Vibrio cholerae*). Bacteria do not have a true nucleus; but the genetic material is located in an irregularly-shaped region called the nucleoid. There is no nuclear membrane or nucleolus.

Structure of DNA (Watson and Crick Model)

The bacterial DNA molecule is composed of two strands of complementary nucleotides that are coiled together in the form of a double helix **(Fig. 6.1)** as described first by Watson and Crick.

- **Each strand is composed of three elements:** It has a backbone of deoxyribose sugar and phosphate groups. The nitrogenous bases are attached to the sugar group. The terms nucleotide and nucleoside are often used to describe the components of the DNA strand:
  - Nucleoside = Sugar + nitrogenous base
  - Nucleotide = Sugar + nitrogenous base + phosphate.
- There are four nitrogenous bases:
  - Two purines—adenine (A) and guanine (G)
  - Two pyrimidines—thymine (T) and cytosine (C).

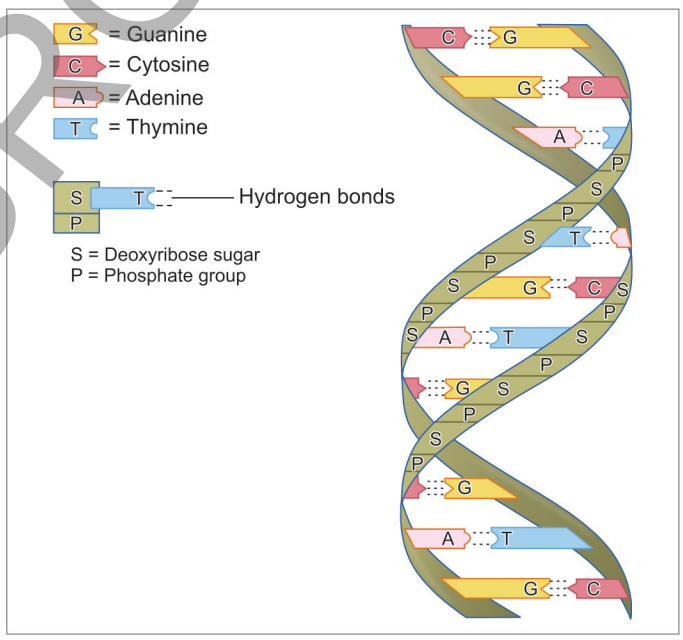

Fig. 6.1: Structure of DNA.

Source: Concept adapted and modified from Jawetz, Melnick & Adelberg's Medical Microbiology; McGraw-Hill Education (*with permission*).

- **Pairing:** The two DNA strands are held together by hydrogen bonds occurring between the nitrogenous bases on the opposite strands. The pairing follows a specific rule:
  - Adenine of one strand binds with thymine (A–T) of other strand by double hydrogen bonds.
  - Guanine of one strand binds with cytosine (G–C) of other strand by triple hydrogen bonds.
- Hence, in a molecule of DNA, the number of adenine molecules is equal to that of thymine, and the number of guanines is equal to cytosines.

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- The ratio of A + T to G + C is constant for each species but varies widely from one bacterial species to another.

BACTERIAL RNA

RNA (ribonucleic acid) is structurally similar to DNA, except for two differences.

1. In sugar—ribose is present instead of deoxyribose.
2. In nitrogenous base—uracil replaces thymine.

There are three different types of RNA in a cell, messenger RNA (mRNA), ribosomal RNA (rRNA) and transfer RNA (tRNA). The main function of RNA is protein synthesis.

POLYPEPTIDE SYNTHESIS

Gene is a segment of DNA that stores information for a particular polypeptide synthesis. The genetic information that is stored in DNA is transcribed into RNA and then translated to form the particular polypeptide.

Transcription

Transcription is a process, in which a particular segment of DNA is copied into RNA by the enzyme RNA polymerase. Since DNA acts as a template for the synthesis of mRNA, therefore, the bases in mRNA are complementary to that of DNA.

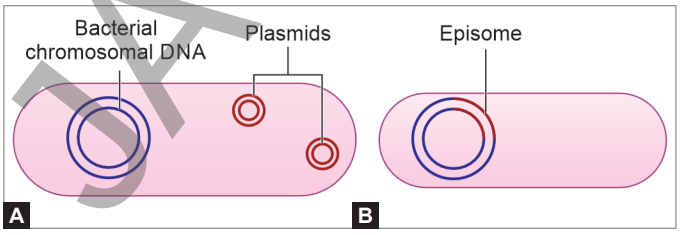
Translation

In translation, the mRNA transcribed from DNA is decoded by ribosomes to produce a specific amino acid chain, or polypeptide. It occurs in the cytoplasm of the bacteria.

PLASMID

Plasmids are the extrachromosomal ds circular DNA molecules that exist in a free state in the cytoplasm of bacteria **(Fig. 6.2A)** and also found in some yeasts.

- **Not essential:** Plasmids are not essential for life; bacteria may gain or lose plasmids during their lifetime.
- **Numbers:** They may be present singly or in multiple numbers—up to 40 or even more per cell.
- **Independent replication:** Plasmids are capable of replicating independently. They can behave as replicons, possessing an origin of replication and other genes that help in replication.
- **Episome:** Sometimes, the plasmid may integrate with the chromosomal DNA of bacteria and such plasmids are called as episomes. They replicate along with the bacterial chromosome **(Fig. 6.2B)**.
- **Curing:** The process of eliminating the plasmids from bacteria is known as curing. It may occur spontaneously or may be induced by treatment of the host cells with substances that inhibit plasmid replication without affecting the host cell, such as acridine dyes, radiation, thymine starvation, and growth at higher temperatures.



Figs. 6.2A and B: Plasmids.

Classification of Plasmids

Plasmids can be classified in many ways:

1. **Based on the ability to perform conjugation:**
   - Conjugative plasmids: Some plasmids have an ability to transfer themselves to other bacteria by means of conjugation. These are called self-transmissible or conjugative plasmids.
   - Non-conjugative plasmids: They are also called as nontransmissible plasmids as they cannot transfer themselves.
2. **Based on compatibility between the plasmids**, they can be grouped into:
   - Compatible plasmids: Different plasmids can exist in a single bacterial cell only if they are compatible to each other.
   - Incompatible plasmids: If two plasmids are not compatible, one or the other will be rapidly lost from the cell. They normally share the same replication or partition mechanisms, hence compete with each other.
3. **Based on function**, there are five main classes of plasmids:
   i. **Fertility or F-plasmids:** They contain *tra*-genes, which code for the expression of sex pili that help in bacterial conjugation by forming the conjugation tube.
   ii. **Resistance (R) plasmids:** They contain genes that code for resistance to various antibiotics.
   iii. **Col plasmids:** They contain genes that code for bacteriocins (antibiotic-like protein substances produced by bacteria that inhibit other bacteria).
   iv. **Virulence plasmids:** They code for certain virulence factors and toxins that help in bacterial pathogenesis. Examples include:
      - Heat-labile and heat-stable toxins of *E. coli*
      - Siderophore production
      - Adherence antigens (K88 plasmid in *E. coli*).
   v. **Metabolic plasmids:** They enable the host in various metabolic activities:
      - Digestion of unusual substances, e.g., toluene and salicylate, camphor, etc.
      - Urease synthesis
      - Nitrogen fixation.

Plasmid as Vector

Plasmids by their ability to transfer DNA from one cell to another, have become important vectors in genetic engineering. Plasmids contain certain sites where genes can be inserted artificially using recombinant DNA technology. Such plasmids can be used for various purposes such as protein production, gene therapy, etc. (described later in this chapter).

GENE TRANSFER IN BACTERIA

Bacteria undergo genetic variation and acquire new genes through a mechanism called mutation. Following which,

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the newly acquired genes are transferred either vertically to their offsprings during cell division or horizontally to other bacteria in the surrounding.

MUTATION

Definition: Mutation is a random, undirected heritable variation caused by a change in the nucleotide sequence of the genome of the cell.

Mutation can involve any of the numerous genes present in bacterial chromosome or rarely plasmid. The frequency of mutation ranges from 10^{-2} to 10^{-10} per bacterium per division. Mutations occur in one of the two ways:

1. **Spontaneous mutations:** Mutations that occur naturally in any dividing cells that arise occasionally without adding any mutagen.
2. **Induced mutations:** These mutations on the other hand, are as a result of exposure of the organism to a **mutagen**, an agent capable of inducing mutagenesis. Examples of mutagens include:
   - Physical agents, e.g. ultraviolet (UV) radiations—cytosine and thymine are more vulnerable to UV rays.
   - Chemical agents, e.g. alkylating agents, 5-bromouracil and acridine dyes.

Mutation is a natural event, taking place all the time, in all dividing cells. Most mutants go unrecognized as the mutation may be lethal or may involve some minor functions that may not be expressed. Mutation is best appreciated when it involves a function, which can be readily observed by experimental methods. For example, *E. coli* mutant that loses its ability to ferment lactose can be readily detected on MacConkey agar.

Mutation can affect any gene and hence may modify any characteristic of the bacterium, for example:

- Sensitivity to bacteriophages
- Loss of ability to produce capsule or flagella
- Loss of virulence
- Alteration in colony morphology
- Alteration in pigment production
- Drug susceptibility
- Biochemical reactions
- Antigenic structure.

The practical importance of bacterial mutation is mainly in the field of drug resistance and the development of live vaccines.

Classification of Mutation Types

Mutations may occur in two ways:

1. **Small-scale mutations:** They are more commonly seen in bacteria. Examples include (1) point mutations—occur at a single nucleotide, (2) addition or deletion of a single nucleotide pair
2. **Large-scale mutations** occur in chromosomal structure: These include deletion or addition of several nucleotide base pairs or gene duplications.

Detection and Isolation of Mutants

Mutation can be recognized by both genetic and phenotypic methods.

- **Gene sequencing:** It is the method of choice currently used.
- **Phenotypic methods:** They are less commonly used now.
  - **Fluctuation test:** It demonstrates spontaneous mutations in bacteria.
  - **Replica plating method:** It is used to demonstrate auxotrophic mutants (unable to grow in the absence of a specific nutrient).
  - **Ames test:** It is used to test the carcinogenicity of a mutagen.

HORIZONTAL GENE TRANSFER

Horizontal gene transfer occurs in bacteria by several methods, such as:

- Transformation (uptake of naked DNA)
- Transduction (transfer through bacteriophage)
- Lysogenic conversion
- Conjugation (plasmid mediated via conjugation tube).

TRANSFORMATION

Definition

Transformation is a process of random uptake of free or naked DNA fragment from the surrounding medium by a bacterial cell and incorporation of this DNA fragment into its chromosome in a heritable form.

Natural transformation has been studied so far only in certain bacteria—*Streptococcus, Bacillus, Haemophilus, Neisseria, Acinetobacter* and *Pseudomonas*.

Mechanism of Transformation

When bacteria lyse, they release large amounts of dsDNA into the surrounding environment, which can be taken up by the bacteria present in the surroundings.

- Some bacteria (e.g. *S. pneumoniae*) that express a transformation promoting factor called **competence factor** can uptake a DNA fragments from other bacteria.
- Whereas some bacteria (*Haemophilus influenzae*) preferentially uptake DNA fragments only from the same or closely related species through species-specific DNA uptake mechanisms.

Griffith Experiment

The famous Griffith experiment (1928) on mice using pneumococci strains provided the direct evidence of existence of transformation.

- Griffith found that mice died when they were injected with a mixture of live noncapsulated pneumococci and heat killed capsulated pneumococci strains. However, neither of which separately proved fatal to mice **(Fig. 6.3)**.
- He stated that the live noncapsulated strains were transformed into the capsulated strains due to transfer of the capsular genes released from the lysis of the killed capsulated strains, which was confirmed later by Avery, Macleod and McCarty in 1944.

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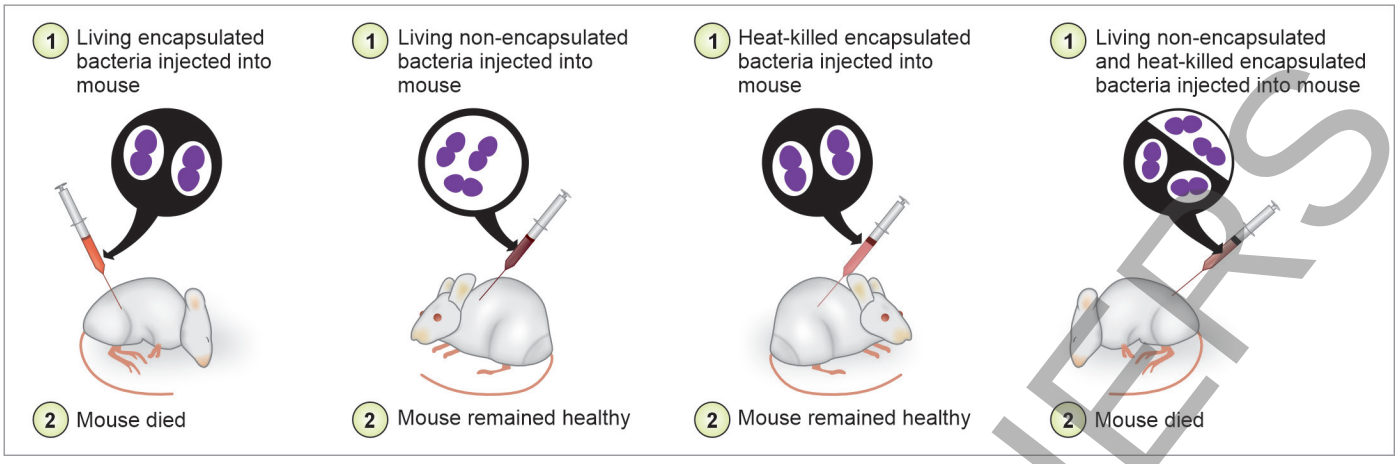

Fig. 6.3: Griffith experiment demonstrating transformation.

TRANSDUCTION

Transduction is defined as transfer of a portion of DNA from one bacterium to another by a bacteriophage.

Mechanism of Transduction

Bacteriophage (or phage) is a virus that infects and multiplies inside the bacterium.

- During transmission of phage from one bacterium to another, a part of the host DNA may accidentally get incorporated into the phage and then gets transferred to the recipient bacterium.
- This leads to acquisition of new characters by the recipient bacterium coded by the donor DNA.

Life Cycles of Phage

After entering a new bacterium, the phage may undergo two types of life cycles.

1. **Lytic or virulent cycle:** The phage multiplies in host cytoplasm, produces a large number of progeny phages, which subsequently are released causing lysis and death of the host cell. In this cycle, inserting a new gene into the bacterium is difficult.
2. **Lysogenic or temperate cycle:** Here, the phage DNA remains integrated with the bacterial chromosome as the prophage, and multiplies synchronously with bacterial DNA.
   - The phage DNA, when transferred to a new bacterium gets disintegrated from the parent bacterial chromosome and in the process, may take up a few bacterial genes.
   - These bacterial genes along with the phage DNA get transferred to a new bacterium.

Types of Transduction

Transduction is of two types, either generalized or restricted.

Generalized Transduction

It involves the transfer of any part of the donor bacterial genome into the recipient bacterium. Generalized transduction usually occurs as a result of defective assembly during the lytic cycle of virulent and some temperate phages.

- Packaging errors may happen occasionally due to defective assembly of the daughter phages. Instead of their own DNA, a part of host DNA may accidentally be incorporated into the daughter bacteriophages.
- The resulting bacteriophage (called transducing phage) often injects the donor DNA into another bacterial cell but does not initiate a lytic cycle as the original phage DNA is lost **(Fig. 6.4)**.

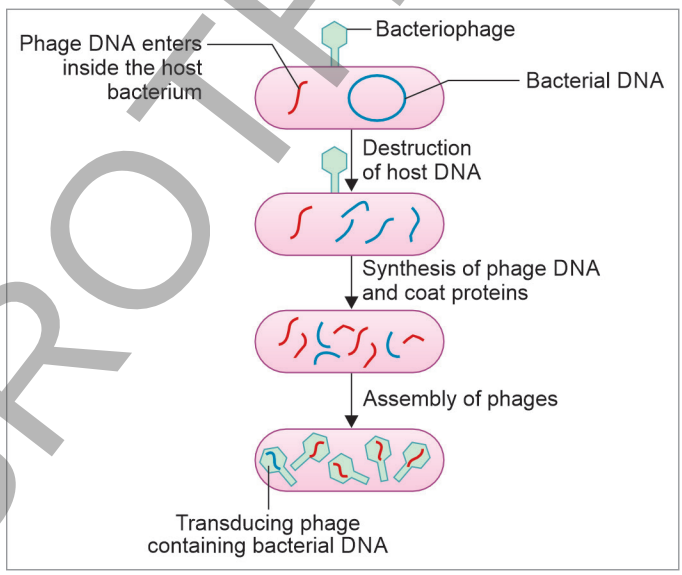

Fig. 6.4: Generalized transduction.

Restricted or Specialized Transduction

In contrast to generalized transduction, restricted transduction is capable of transducing only a particular genetic segment of the bacterial chromosome that is present adjacent to the phage DNA. It occurs as a result of a defect in the disintegration of the lysogenic phage DNA from the bacterial chromosome.

- When a prophage (i.e. lysogenic bacteriophage is integrated with the bacterial chromosome) leaves the host chromosome, portions of the bacterial chromosome present adjacent to the phage DNA may get wrongly excised along with it.
- Such transducing phages carrying a part of bacterial DNA in addition to their DNA, when infect another bacterium, the transfer of the donor DNA takes place **(Fig. 6.5)**.

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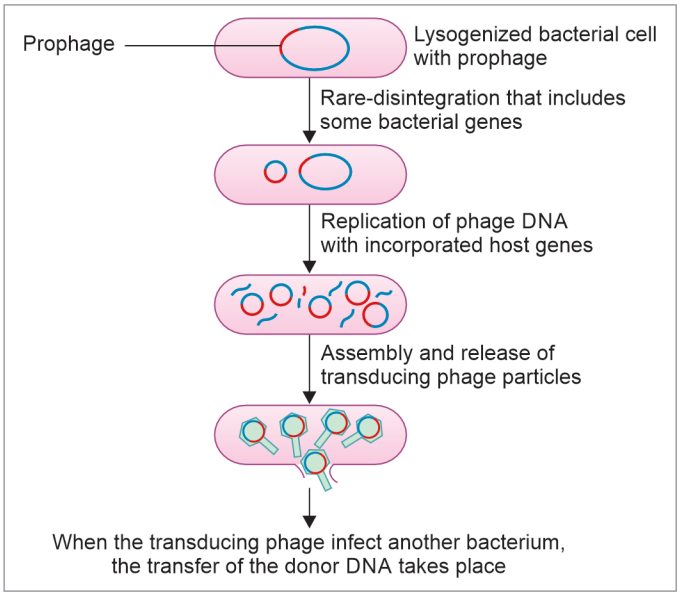

Fig. 6.5: Restricted transduction.

Role of Transduction

In addition to chromosomal DNA, transduction is also a method of transferring episomes and plasmids.

- **Drug resistance:** Transduction may be a mechanism for the transfer of bacterial genes coding for drug resistance; for example, plasmid coded penicillin resistance in staphylococci.
- **Treatment:** Transduction has also been proposed as a method of genetic engineering in the treatment of some inborn metabolic defects.

LYSOGENIC CONVERSION

Here, the phage DNA which is integrated into the host bacterial chromosome (during the lysogenic cycle), itself codes for several virulence factors such as toxins. When such bacteriophage infects a new host, the virulence genes also get transferred.

- The prophage acts as an additional chromosomal element that encodes for new characters and is transferred to the daughter cells. This process is known as lysogeny or lysogenic conversion.
- **Imparts toxigenicity to the bacteria:** Phage DNA may be responsible for bacterial virulence by coding for their toxin production. For example, in *Corynebacterium diphtheriae*, the diphtheria toxin is coded by a lysogenic phage DNA which is integrated with the bacterial chromosome. Elimination of the phage from a toxigenic strain renders the bacterium nontoxigenic.

Phage Coded Toxins

Bacterial toxins that are coded by lysogenic phages include:

- ❑ Diphtheria toxin
- ❑ Cholera toxin
- ❑ Verocytotoxin of *E. coli*
- ❑ Streptococcal pyrogenic exotoxin (SPE)—A and C
- ❑ Botulinum toxin types C and D

- In lysogenic conversion, the phage DNA itself behaves as the new genetic element, in contrast to transduction where the phage acts only as a vehicle carrying bacterial genes.

CONJUGATION

Conjugation refers to the transfer of genetic material from one bacterium (donor or male) to another bacterium (recipient or female) by mating or contact with each other and forming the conjugation tube. It was discovered first by Lederberg and Tatum (1946).

F^+ X F^- Mating

The F^+ cell (also called as the donor or the male bacterium) contains a plasmid called F factor or fertility factor. The bacteria lacking the F factor are called as recipient or female bacteria or F^- cells.

- F factor is a conjugative plasmid; carries genes that encode for the formation of sex pilus (which helps in conjugation) and self plasmid transfer.
- The F pilus brings the donor and nearby recipient cells close to each other and forms a conjugation tube that bridges between the donor and recipient cells **(Fig. 6.6)**.

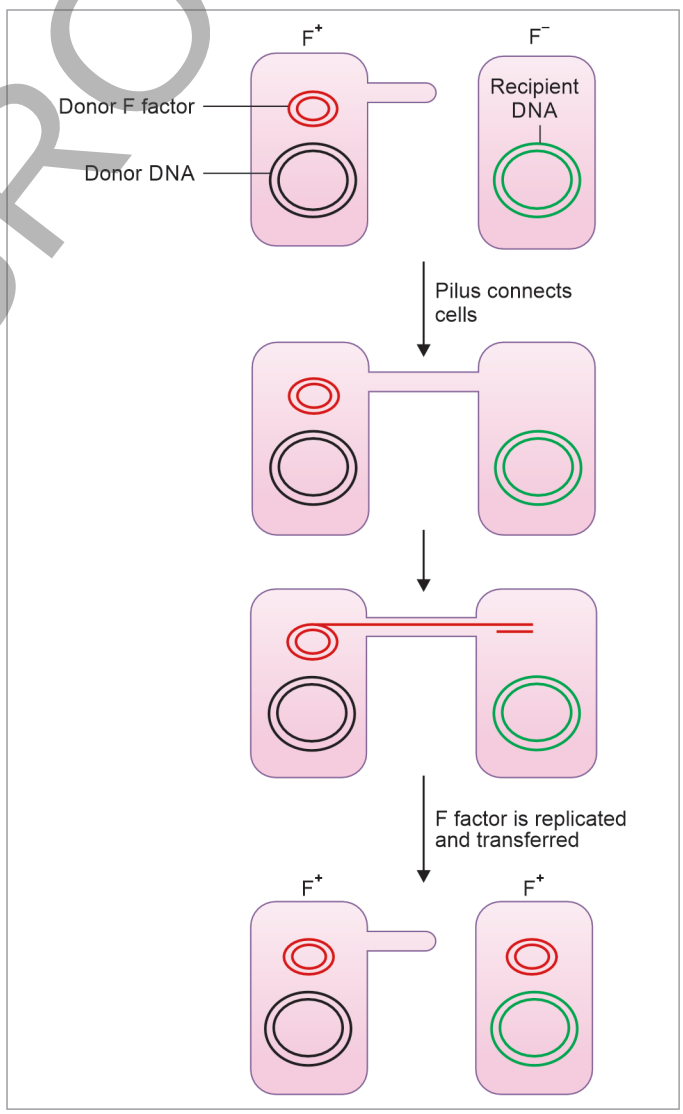

Fig. 6.6: Bacterial conjugation (F^+ X F^- mating).

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- During conjugation, the plasmid DNA replicates by the rolling-circle mechanism, and a copy moves to the recipient bacterium through the conjugation tube. Then, in the recipient, the entering strand is copied to produce a complete F factor with ds DNA.
- As a result, the recipient (F^-) becomes (F^+) cell and can in turn conjugate with other (F^-) cells. Therefore, it is said that this character of maleness (F^+) in bacteria is transmissible or infectious.
- During F^+ X F^- conjugation, chromosomal genes from donor bacterium may rarely be transferred along with F factor. Here, though the donor chromosomal gene may undergo recombination with the recipient chromosome; but with a lower frequency.

HFR Conjugation

F factor being a plasmid, it may integrate with bacterial chromosome and behave as episome.

- Such donor cells are able to transfer chromosomal DNA to recipient cells with high frequency in comparison to F^+ cells, therefore, named as Hfr cells (high frequency recombination).
- During conjugation of Hfr cell with an F^- cell, only a few chromosomal genes along with a part of the F factor get transferred. Connection between the cells usually breaks before the whole genome is transferred.
- As the entire F factor does not get transferred, hence following conjugation, F^- recipient cells do not become F^+ cells.

F′ Conjugation

The conversion of a F^+ cell into a Hfr cell is reversible.

- When the F factor reverts from the integrated to free-state, it may sometimes carry with it some chromosomal DNA from the adjacent site of its attachment. Such an F factor carrying some chromosomal DNA is named as F' factor (F prime factor).
- When F' cell conjugates with a recipient (F^-), it transfers the host DNA incorporated with it along with the F factor. The recipient becomes F' cell. This process is called sexduction.

Role of Conjugation

- Conjugation plays an important role in the transfer of plasmids coding for antibacterial drug resistance [resistance transfer factor (RTF), see the box in the preceding text] and bacteriocin production [Colicinogenic (Col) factor].
- Conjugation is the most common mechanism of transfer of plasmids coding for multiple drug resistance among bacteria. Several drug resistance genes can be transferred together along with the F factor, which is the main reason for the emergence of **multi-drug resistance** in bacteria.

Role of Conjugation in Bacterial Drug Resistance

Conjugation is also an important method of transfer of plasmids coding for multiple drug resistance among bacteria. **R factor** (or the resistance factor) is a plasmid that has two components.

Contd...

Contd...

1. Resistance transfer factor (RTF): It is the plasmid responsible for conjugational transfer (similar to F factor).
2. Resistance determinant (r): R factor can have several r determinants and each r determinant codes for resistance to one drug.

Mutational and transferable drug resistance have been discussed in Chapter 7.

Fate of the Donor DNA

Following horizontal gene transfer, the donor DNA either gets degraded by host nucleases or may integrate with recipient chromosome by a method called **recombination**. Donor DNA integrates into the recipient chromosome either as a replacement piece (commonly seen in transformation) or as an additional piece (seen in other methods of gene transfer).

TRANSPOSITION

Transposons or transposable elements are the bacterial genes that are capable of intracellular transfer between chromosome to chromosome, plasmid to plasmid, and chromosome to plasmid or vice versa and the process of such intracellular transfer of transposons is called as transposition. As transposons move around the genome in a cut-and-paste manner, they are also called **jumping genes** or **mobile genetic elements**.

- Transposition does not require any DNA homology between transposon and the site of insertion. It is, therefore, different from recombination.
- Unlike plasmids, transposons are not self-replicating and are dependent on chromosomal or plasmid DNA for replication.
- Transposons were first discovered in the 1940s by Barbara McClintock during her studies on maize genetics for which she won the Nobel Prize in 1983.
- Transposons are also discovered in the virus and in eukaryotic genome.

Types of Transposons

Insertion Sequence Transposon

The simplest form of transposon is an insertion sequence. It is about 1–2 kilo basepairs (kbp) in length and consists of a **transposase gene** (that helps in transposition) which is flanked at both the ends by **inverted repeat sequences** of nucleotides, i.e. nucleotide sequences complementary to each other but in the reverse order **(Figs. 6.7A and B)**.

Because of this feature, each strand of the transposon can form a single-stranded loop carrying the transposase gene, and a double-stranded stem formed by hydrogen bonding between the terminal inverted repeat sequences.

Composite Transposon

They are larger transposons carrying additional genes, such as genes coding for antibiotic resistance or toxin production in the center and both ends are flanked by insertion sequences that are identical or very similar in sequence **(Fig. 6.7C)**.

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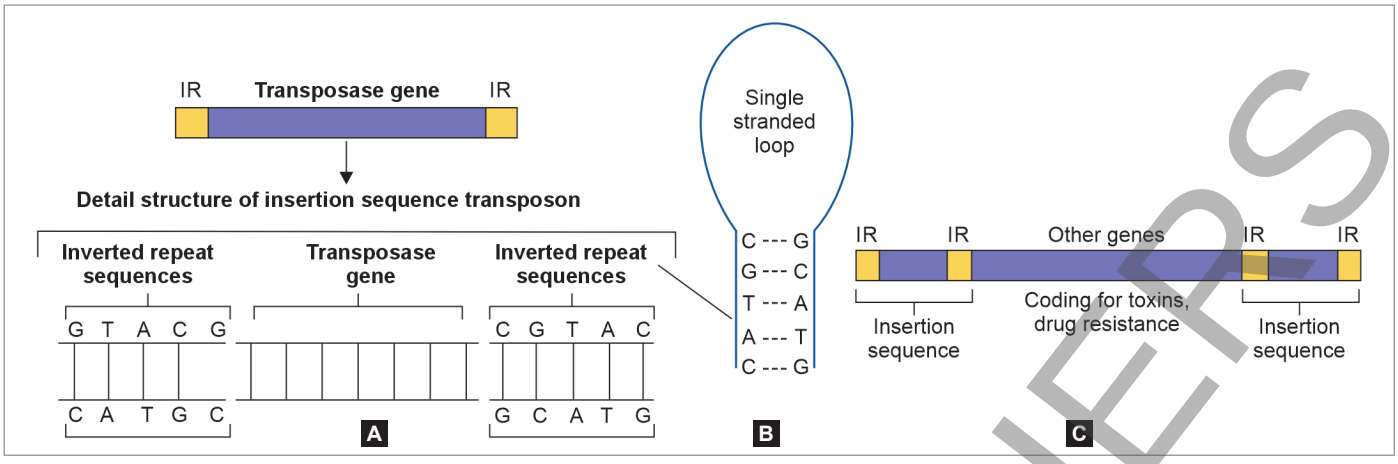

Figs. 6.7A to C: Transposons: (A) Insertion sequence transposon; (B) Hairpin loop structure of transposon; (C) Composite transposon.

GENE TRANSFER BY ARTIFICIAL METHODS

GENETIC ENGINEERING

Genetic engineering refers to deliberate modification of an organism's genetic information by directly altering its nucleic acid genome. Genetic engineering is accomplished by a precise mechanism known as recombinant DNA technology.

The gene coding for a desired protein is isolated from an organism, and then inserted into suitable vector, which is then cloned in such a way that it can be expressed in the formation of specific (desired) protein.

Recombinant DNA Technology

The procedure of recombinant DNA technology involves the following steps:

1. **Treatment with restriction enzyme:** The DNA from the microorganism is extracted and then is cleaved by enzymes called restriction endonucleases to produce a mixture of DNA fragments.
2. **Southern blot:** The fragment containing the desired gene is isolated from the mixture of DNA fragments. This is done by:
   - Electrophoresis: DNA fragments are electrophoretically separated by subjecting to agar gel electrophoresis.
   - Transfer to nitrocellulose membrane: The separated DNA fragments are transferred from the gel to a nitrocellulose membrane.
   - Detection of the desired gene: The DNA fragment containing the desired gene is detected adding a specific DNA probe, complementary to the gene of interest.
   - Isolation: The band containing the desired gene is isolated by DNA extraction and then, is subjected to electrophoresis in a different gel.
3. **Recombination with a vector:** The isolated DNA fragment is annealed with a vector by DNA ligase enzyme.
4. **Introduction of the vector into bacteria:** The vector is introduced into bacteria usually by transformation (injecting by electroporation) and rarely by phage vector by transduction.
5. **Cloning:** Culture of the bacteria containing the desired gene followed by expression of the gene products, yields a large quantity of desired protein.

Applications of Genetic Engineering

- **Production of vaccines:** Protein antigens prepared using DNA recombinant technology are used as immunogens against which protective antibodies are produced, e.g, subunit vaccines for hepatitis B virus and papillomavirus.
- **Production of antigens used in diagnostic kits:** Used for specific antibody detection (e.g. ELISA) are prepared by DNA recombinant technology.
- **Production of proteins used in therapy:** These include human growth hormone, insulin, interferons, interleukin-2, tumor necrosis factor, and factor VIII.
- **Transgenic animals:** Recombinant DNA technology can be used to artificially introduce a foreign DNA into the genome of animals. The process is called transfection and the recombinant animals produced in this way are named transgenic or genetically modified animals. Transgenic mice are available for a variety of biotechnological applications.
- **Gene therapy:** Genetic diseases can be treated by replacing the defective gene by introducing the normal gene into the patient.

Vector

A vector is a small piece of DNA, into which a foreign DNA fragment can be inserted and that can be stably maintained in an organism and used for cloning purposes. There are four major types of vectors, such as: (1) Plasmids, (2) bacteriophages, (3) cosmids, (4) artificial chromosomes, such as bacterial/yeast artificial chromosomes.

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BLOTTING TECHNIQUES

A blot, in molecular biology refers to a method of transferring DNA, RNA, or proteins, from the gel onto a carrier (e.g. nitrocellulose membrane), followed by their detection by using specific nucleic acid probes (for DNA or RNA detection) or enzyme immunoassay (for protein detection).

There are various blotting techniques:

- ❖ Southern blot is used to detect DNA
- ❖ Northern blot is used to detect RNA
- ❖ Western blot is used to detect proteins
- ❖ Eastern blot: It is a modification of Western blot, used to analyze proteins for post-translational modifications using probes that may detect lipids, carbohydrates, phosphorylation or any other protein modification.

Southern blotting technique is described above, under genetic engineering. The methodology of Northern blot is similar to Southern blot, except that RNA is transferred and detected using a labeled complementary nucleic acid probe. Western blot is described in Chapter 14.

EXPECTED QUESTIONS

I. Write essay on:

1. Name various methods of horizontal gene transfer. Discuss in detail about mechanism of conjugation.

II. Write short notes on:

1. Mutation
2. Transformation
3. Transposition
4. Transduction

III. Multiple choice questions (MCQs):

1. Mechanism of direct transfer of free DNA:
a. Transformation b. Conjugation
c. Transduction d. Transposition

2. Phage mediated transfer of DNA from one bacterium to another bacterium is known as:
a. Transformation
b. Transduction
c. Transmission
d. Conjugation

3. Horizontal transmission of 'R' factor is by:
a. Transduction b. Transformation
c. Conjugation d. Fusion

4. F factor carrying chromosomal DNA is called:
a. F factor b. Hfr
c. RTF d. F′ factor

5. Sometimes, the plasmid may integrate with chromosomal DNA of bacteria and such plasmids are called:
a. Episomes b. Transfer factors
c. Vectors d. Cosmids

6. NOT a method of horizontal gene transfer is:
a. Conjugation
b. Mutation
c. Transformation
d. Transduction

7. The Griffith experiment on mice using pneumococcal strains provides the direct evidence of:
a. Transformation
b. Transduction
c. Conjugation
d. Transposition

8. Competence factor-mediated transformation occurs in:
a. *Haemophilus*
b. *Neisseria*
c. *S. pneumoniae*
d. *Pseudomonas*

9. Which of the following bacterial toxin is not phage coded toxin?
a. Diphtheria toxin
b. Verocytotoxin of *E. coli*
c. Botulinum toxin C and D
d. Heat-labile toxin of *E. coli*

10. A donor strain of *E. coli* transfers chromosomal markers to a recipient at high frequency, but the complete F factor is rarely transferred. The donor strain is most likely:
a. F– strain
b. F+ strain
c. F′ strain
d. Hfr strain

Answers

1. a **2.** b **3.** c **4.** d **5.** a **6.** b **7.** a **8.** c **9.** d **10.** d

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CHAPTER 10

Epidemiology of Infectious Diseases

CHAPTER PREVIEW

- Infection and Related-terminologies
- Epidemiological Patterns
- Eradication and Elimination
- Epidemiological Determinants of Disease Causation
  - Agent Factors
  - Host Factors
  - Environmental Factors

The epidemiology branch of infectious disease deals with the distribution and determinants of infection-related health state in specified populations, and its application to the control of the disease.

INFECTION AND RELATED-TERMINOLOGIES

Following the entry of the microorganism into the body, it may lead to either infection or colonization; both the terms need to be distinguished.

- **Infection:** It is a process in which a pathogenic organism enters, establishes itself, multiplies and invades the normal anatomical barrier of the host resulting in disease. When infection becomes apparent results in clinical manifestation and it is referred to as 'infectious disease.'
- **Colonization:** Here, the pathogenic organism enters, multiplies but does not invade, and neither causes disease nor elicits a specific immune response.
  - Colonizers are different from normal flora. They have pathogenic potential and may invade and cause disease in another host or the same host later.
  - However, commensals are limited to a particular anatomical site, e.g. intestine, respiratory and genital tract. When they enter through other routes, they may behave as pathogens. For example, *Escherichia coli* is a normal flora in the intestine, but it causes infection when enters through respiratory or urinary tract.
- **Infestation:** It refers to inhabiting the surface of the body of the host by certain larger parasites called as **ectoparasites**; without penetrating into the tissues.
  - They act as important vectors to transmit the pathogenic microbes—such as louse, fleas, mites and ticks, etc. (Chapter 73, Ectoparasites).
  - Some of these ectoparasites may penetrate into the host causing disease; examples *Sarcoptes scabiei* causing scabies (Chapter 73, Ectoparasites).

The infectious diseases can also be classified based on being developed in community or in hospital.

- **Healthcare-associated infection (HAIs):** Defined as, the new infections acquired in a healthcare facility (HCF) by a patient after 48 hours of admission, which was neither present nor incubating at the time of admission (refer to Chapter 23 for details).
- **Community-associated infections:** Refers to the infections which developed in the community or within 48 hours of admission to a HCF.

EPIDEMIOLOGICAL PATTERNS

Infectious diseases that are capable of directly transmitting to man from another man, animal or environment (air, water, food, etc.) are called as communicable disease. The spread of communicable diseases in the community may occur in several epidemiological patterns—outbreak, epidemic, pandemic, hyperendemic and sporadic.

- **Outbreak** is a sudden rise in the number of cases in a limited geographic area. If it's not quickly controlled, an outbreak can become an epidemic. Examples include:
  - Cholera outbreak in Bengaluru in 2020, affecting 17 people.
  - Nipah virus encephalitis outbreak in Kerala in 2018, resulting in 18 cases with 16 deaths.
- **Epidemic:** If the infection occurs at a much higher rate than usual in a particular geographical area, it is known as epidemic. It usually affects a large number of people within a community, population, or region. If it is not quickly contained, it can result in a pandemic. The classical examples include:
  - SARS epidemic in China in 2003.
  - Ebola epidemic in Africa in 2014.
  - Zika epidemic in Brazil in 2015.
- **Pandemic:** Infection that spreads rapidly to large areas of the world is known as pandemic. Examples include:
  - COVID-19 pandemic in 2020 affecting >200 countries.
  - Influenza pandemics: Several flu pandemic occurred so far including H1N1 pandemic in 2009.

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- Cholera: Seven pandemics of cholera have occurred so far in the past.
- Plague: Three pandemics occurred so fast in the past. The second pandemic (black death) occurred in 14th century, killed over 50 million of mankind.

❖ **Endemic:** When a disease occurs at a persistent, usually low level in a certain geographical area, it is called as endemic. India is endemic for several diseases such as typhoid fever, cholera, filariasis, malaria, etc.

❖ **Hyperendemic:** When a disease occurs at persistent high level in a geographical area, it is known as **hyperendemic**. Examples include:
- India is hyperendemic for hepatitis A and E.
- India was hyperendemic for polio until 1990. With constant immunization effort, India was declared polio-free in 2014.

❖ **Sporadic:** Infections occur at irregular intervals or only in a few places; scattered or isolated. For example, several sporadic cases of cholera occur in India every year.

ERADICATION AND ELIMINATION

Eradication, elimination and control of an infectious disease are related terminologies with distinct differences.

Eradication

It refers to the complete and permanent worldwide reduction to 'zero new cases' of the disease through deliberate efforts. If a disease has been eradicated, no further control measures are required.

❖ Smallpox was the only disease to be eradicated from the whole world (in 1980).

❖ Polio is on the verge of eradication. Most countries, including India, have already been declared polio-free; endemic transmission persists only in Pakistan and Afghanistan.

❖ Guinea worm disease has been eliminated from most countries and remains endemic in only a few countries in sub-Saharan Africa.

Elimination

It refers to the 'reduction to zero' (or a very low defined target level) of new cases in a defined geographical area. Elimination requires continued measures to prevent re-establishment of disease transmission. Diseases that have achieved elimination targets in India include neonatal tetanus and leprosy.

Control

It refers to the reduction of disease incidence, prevalence, morbidity or mortality to a locally acceptable level as a result of deliberate efforts. However, continued intervention measures are still required to maintain the reduction, e.g. diarrheal diseases.

EPIDEMIOLOGICAL DETERMINANTS OF DISEASE CAUSATION

The **Epidemiological Triad** is one of the traditional, but simplest model for depicting causation of infectious disease. The triad consists of an external **agent**, a susceptible **host**, and an **environment** that brings the host and agent together.

AGENT FACTORS

It refers to the infectious microorganism such as a virus, bacterium, parasite, or fungus that is responsible for the causation of the disease. A variety of agent-related factors influence whether the exposure to an organism will result into disease **(Table 10.1)**.

Organism's Pathogenicity

Pathogenicity refers to the ability of the organism to cause disease. Only the pathogenic organisms are capable of producing the disease. This is because they express various virulence factors that allow the organism to become established in a host and maintain the disease state.

Infective Dose

Infective dose is the minimum inoculum size that is capable of initiating an infection.

❖ **Low infective dose**: e.g. *Shigella, Cryptosporidium parvum, E. coli* O157: H7, *Giardia* and *Campylobacter jejuni*. They require small inoculum to initiate infection.

❖ **Large infective dose:** In contrast, organisms with a high infective dose can initiate the infection only when the inoculum size exceeds a particular critical size. For example, include *Salmonella* and *Vibrio cholerae.*

Source and Reservoir

The starting point for the occurrence of an infectious disease is known as a source or/and reservoir of infection. They are not always synonymous.

❖ **Source:** It refers to a person, animal, or object from which the microorganism is transmitted to the host.

❖ **Reservoir:** It is the natural habitat in which the organism lives and multiplies. It may be a person, animal,

Table 10.1: Epidemiological determinants of disease causation.

Agent
• Organism's pathogenicity • Infective dose • Source and reservoir: Human or animal • Mode of transmission: Contact, inhalation, ingestion, vector-borne, vertical transmission • Infectivity or communicability
Host
• Age, gender, and race • Underlying disease, pregnancy, etc. • Underlying immune status and nutritional status • Occupational status • Personal practices: Hygiene and sexual practices • Genetic make-up
Environment
• Seasonality • Resistance to disinfectants • Effective antimicrobial agents • Soil, moisture, oxygen, rainfall

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arthropod, plant, soil or substance (or combination of these) on which the organism depends for its survival and multiplication, and from which it can be transmitted to a susceptible host.

The term source and reservoir may be the same for many organisms but are not always identical. For example:

- In tetanus infection, the reservoir and source of the agent *(Clostridium tetani)* are same, i.e. the soil.
- In hookworm infection, the reservoir is man, but the source of infection is the soil contaminated with hookworm larvae.
- In typhoid fever, the reservoir may be a case or carrier, but the source of infection is usually contaminated food or water.

Thus, the term 'source' refers to the immediate source of infection and may or may not be a part of reservoir.

The reservoir (and/or source) may be of three types.

Human Reservoir

By far the most important reservoir and/or source of infection for humans is man himself. Man is often described as his own enemy because most infectious diseases are contracted from human sources. The diseases that can be spread from one person to another are called **communicable diseases**. Human sources may be either cases or carriers.

- **Cases or patients:** They are persons in a given population identified as having a particular disease.
- **Carrier:** It refers to persons/animals who harbor the infectious agent in the absence of any clinical symptoms and shed the organism from the body via contact, air or secretions.
  - It results from inadequate treatment or immune response, which leads to incomplete elimination of the organism from the body.
  - Though, carriers are less infectious than cases, they are more dangerous as they often go undetected and continue to transmit the infection for a long period.

Types of Carriers

Carriers can be of various types:

- **Incubatory carriers** are those who shed the organism during the incubation period of the disease. This usually occurs in the last few days of incubation period*, e.g. measles, mumps, polio, diphtheria, pertussis, hepatitis B, influenza, etc.
- **Healthy carriers** refer to the subclinical cases who develop into carriers without suffering from overt disease, e.g. polio, cholera, salmonellosis, diphtheria, meningococcal meningitis, etc.
- **Convalescent carrier** is one who has recovered from the disease and continues to harbor and shed the pathogen from the body.

Depending on the duration of carriage:

- **Temporary carriers:** They shed the organisms for less than six months. Incubatory, healthy and convalescent carriers are actually the types of temporary carriers.
- **Chronic carriers:** They shed the organisms for an indefinite period, e.g. in hepatitis B, typhoid fever, etc.

Contd...

Contd...

Depending on the source:

- Contact carrier is a person who acquires the pathogen from a patient.
- Paradoxical carrier refers to a carrier who acquires the pathogen from another carrier.

***Note:** Incubation period (IP) is the time between the entry of an infectious agent (e.g. virus or bacteria) into the body and the appearance of the first symptoms of the disease.

Animal Reservoir

The source of infection may sometimes be animals and birds. Diseases and infections that are transmitted to humans from vertebrates are called zoonoses. Common examples include:

- **From animals:** Rabies (from dogs), leptospirosis (from rodents), influenza (from pigs), etc.
- **Birds** may be a source of infection for various diseases like influenza, *Chlamydia psittaci* infection (psittacosis), histoplasmosis, etc.
- **Amplifying host:** It refers to the vertebrate reservoir in which the organism multiplies exponentially, e.g. pigs in Japanese B encephalitis.

Non-living Things as Reservoir

Soil and inanimate matter can also act as reservoir/source of infection, for example, soil may harbor the agents of tetanus, anthrax and some intestinal helminths such as *Trichuris*, hookworm and *Ascaris*.

Mode of Transmission

Microorganisms may be transmitted from the reservoir or source to a susceptible host in different ways.

Contact

This is the most common mode of transmission. Infection may be transmitted by direct or indirect contact.

- **Direct contact** is via skin and mucosa of an infected person, e.g. through an unclean hand, kissing, or sexual contact. Organisms transmitted by direct contact include agents of common cold, skin and eye infections and agents of sexually transmitted infections (STIs), such as HIV, *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and *Treponema pallidum*, etc.
- **Indirect contact** is through the agency of fomites, which are inanimate objects, such as clothing, toys, etc. These may be contaminated by a pathogen and act as a vehicle for its transmission, e.g. face towels shared by various persons may lead to spread of trachoma.

Inhalation

Inhalational route is the second most common mode of transmission. Transmission through respiratory route occurs either through droplets or aerosols.

Droplet Transmission

Respiratory droplets are large-particles (>5 μm in size) generated during coughing, sneezing or talking.

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- Transmission via large droplets requires close contact [typically within 1 meter (3 feet)], as the droplets do not remain suspended in the air and generally travel only for short distances.
- Along with droplet mode, there is also possibility of contact transmission as droplets may fall on surfaces and fomites present within 1 meter. People can subsequently acquire infection when they touch the infected surfaces or fomites and then touch their nose, eyes or mouth.
- Agents transmitted through droplets include:
  - Bacterial agents/diseases: Diphtheria, *H. influenzae*, meningococcus, pertussis, pneumonic plague, streptococcal pharyngitis.
  - Viral agents/diseases: COVID-19, influenza, viral hemorrhagic fever (e.g. Ebola), mumps, parvovirus B19, rhinovirus, rubella, adenovirus.

Aerosol Transmission

Aerosols are small-particles (<5 μm) generated by an infectious person during coughing, sneezing, talking or while performing certain aerosol generating procedures (e.g. intubation). These smaller droplets remain suspended in air for long periods of time and may disperse to a distant place along the air current.

Infectious agents that are transmitted through aerosols include:

- *Mycobacterium tuberculosis*
- Measles virus
- Varicella (chickenpox and zoster).
- Smallpox (variola) virus.

Ingestion

Infectious agents can be transmitted by the ingestion route, either through contaminated water or food. Food-borne infections occur mostly through carriers engaged in handling or preparation of food and contaminating the foodstuffs. The water supply may get contaminated with the feces of patients or carriers. Examples include:

- Intestinal infections like cholera, dysentery, diarrheagenic *E. coli* and intestinal parasitic infections and viral agents of gastroenteritis, such as rotavirus.
- Extraintestinal infections: In this type of infection, pathogens are transmitted by the enteric route but produce disease manifestations elsewhere—*Salmonella* Typhi (typhoid fever), hepatitis A and E viruses, poliovirus, *Echinococcus granulosus* (causing hydatid disease) and *Taenia solium* (causing cysticercosis).

Inoculation

Pathogens, in some instances, may be inoculated directly into the skin or tissues of the host:

- **Animal bite**—for example, rabies virus is inoculated directly by the bite of a rabid animal
- **Inoculated directly into tissue**—spores of *Clostridium tetani* present in soil may get deposited directly into the host tissues following severe wounds leading to tetanus.

Transmission of Blood-borne Infections

Blood-borne infections, such as hepatitis B, hepatitis C and HIV may be transmitted by:

- Needle prick and other sharp injuries
- Blood transfusion
- Intravenous drug abuse (sharing contaminated needles or syringes).

Vector Borne

Arthropod vectors, such as mosquitoes, flies, fleas, ticks, mites, and lice are the vectors that transmit many diseases (Chapter 73, Ectoparasites). Vectors may be of two types:

- **Mechanical vectors:** These carry the microorganisms (do not multiply) and transmit them to the eatables.
- **Biological vectors:** The pathogen multiplies in the body of the vector, often undergoes part of its developmental cycle in it, such vectors are named as biological vectors (e.g. female *Anopheles* mosquito in malaria; *Culex* mosquito in bancroftian filariasis).

Extrinsic incubation period: After the entry of pathogen into the vector, the time required for the vector to become infective is called the extrinsic incubation period.

Vertical Transmission

It refers to the transmission of infection from mother to the fetus. It may be categorized into:

- **Transplacental transmission:** Infection transmitted via the placental barrier can lead to abortion, miscarriage or stillbirth. If babies are born, they suffer from congenital malformations; such infections are known as teratogenic infections. The pathogens causing congenital infections are abbreviated as **'TORCH'**:
  - **T***oxoplasma gondii*
  - **O**thers (*Treponema pallidum,* varicella-zoster virus, parvovirus, zika virus)
  - **R**ubella virus
  - **C**ytomegalovirus
  - **H**erpes simplex virus.
- **Transmission via the birth canal or during the perinatal period,** usually without causing congenital malformation in the baby, Examples include: Group B *Streptococcus, Neisseria gonorrhoeae* and *Chlamydia trachomatis, Listeria* and viruses (e.g. hepatitis B, C and HIV).

Infectivity or Communicability

It refers to ability of an infectious agent to transmit from one person to another. **Period of communicability** or period of infectivity is the time during which an infectious agent may be transferred directly or indirectly from an infected person to another person.

- Chickenpox: From -2 to +5 days of onset of rash (until all lesions are crusted)
- Mumps: From -2 to +5 days of onset of parotitis
- Measles: From -4 to +4 days of onset of rash
- Rubella : From -1 to +1 week of onset of rash
- Influenza: From -1 to +5-7 days of onset of symptoms
- COVID-19: From -2 to +10 days of onset of symptoms.

HOST FACTORS

Host refers to the human who can get the disease. A variety of factors intrinsic to the host, sometimes called risk factors,

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can influence an individual's exposure, susceptibility, or response to a causative agent.

- **Age:** Most viral infections are common at extremes of age, i.e. childhood and old age. However, sexually transmitted viral infections are common among young adults.
- **Gender:** Most infections are either equally distributed or more common in males. Sex differences in infectious diseases in humans may be due to social, behavioral and sexual practices and may also be due to genetic factors.
- **Pregnancy:** Certain diseases are common in pregnancy such as transplacental infections (e.g. CMV, rubella) or infection through the birth canal such as *S. agalactiae* infection.
- **Host immune status:** Low immunity predisposes to many infections, such as CMV. On the other hand, certain infections require an adequate host immune response (e.g. dengue hemorrhagic fever).
- **Prior immunity:** Prior immunity to the agent due to vaccination or past infection can protect the individual from further infection. Some viral infections such as smallpox, chickenpox, measles, mumps, and rubella provide lifelong immunity.
- **Nutritional status:** Malnutrition lowers the host immunity and thus predisposes to many viral infections, e.g. measles. However, in certain viral infections such as dengue, malnutrition has a paradoxical effect.
- **Underlying comorbid disease**: People with diabetes, immunodeficiency disorders or receiving steroid therapy are more prone to acquire various infections.
- **Occupational status:** Sometimes, infectious diseases are more common in certain occupations; for example, zoonotic diseases such as anthrax are common among butchers, abattoir workers, and farmers.
- **Racial susceptibility:** Sometimes, innate immunity is confined to a particular race; may be absent in other communities. For example, African Americans are more susceptible to tuberculosis than whites.
- **Sexual practices:** People with multiple sex partners and men who have sex with men are more prone to develop various sexually-transmitted infections such as HIV.
- **Hygiene:** Poor hygiene, poor sanitation, over-crowding, etc. predispose to several diseases such as acute diarrheal illness and typhoid fever.
- **Genetic makeup:** Certain individuals are more prone to develop some microbial infections. This depends on the genetic makeup of the individual.

ENVIRONMENTAL FACTORS

Environmental factors play an important role in disease causation.

- **Seasonality:** Many diseases are common in winter such as influenza and meningococcal meningitis; whereas vector-borne diseases such as malaria and dengue are more common in rainy season which parallels mosquito breeding. Leptospirosis is more common in rainy season.
- **Disinfectants:** The organisms which are more resistant to the action of disinfectant can survive in the environment for longer. This is particularly important in the hospital environment, where the multidrug resistant organisms such as *Pseudomonas, Acinetobacter* and *Klebsiella,* etc. are widely prevalent.
- **Soil:** Damp, sandy or friable soil with vegetation is suitable for certain soil-transmitted helminths such as hookworm, *Ascaris* and *Trichuris* than clay soil.
- **Moisture:** Moisture is necessary for the survival of most microbes as dryness is rapidly fatal.
- **Presence of vectors:** Vector-borne diseases have a strict geographical preponderance, depending upon the presence of vector (refer to Chapter 73 for details).
  - Malaria (*Anopheles*) is more common in Africa and India than the rest of the world.
  - Arboviruses are geographically restricted; for example, dengue (*Aedes*) and Japanese encephalitis (*Culex*) are common in India.

EXPECTED QUESTIONS

I. Write short notes on:
1. Epidemiological patterns.
2. Types of carriers.
3. Droplet versus aerosol transmission.

II. Multiple choice questions (MCQs):

1. If the infection occurs at a much higher rate than usual in a particular geographical area, it is known as:
a. Epidemic b. Pandemic
c. Hyperendemic d. Sporadic

2. Organisms with low infective dose include all, *except*:
a. *Shigella*
b. *Cryptosporidium parvum*
c. *Giardia*
d. *Vibrio cholerae*

3. Aerosol transmission occurs in all, *except*:
a. *Mycobacterium tuberculosis*
b. Meningococcus
c. Measles virus
d. Varicella (chickenpox and zoster)

4. Infection that spreads rapidly to large areas of the world is known as___.
a. Outbreak b. Pandemic
c. Epidemic d. Endemic

5. The person who harbors the infectious agent in the absence of any clinical symptoms and sheds the organism to others is called_____.
a. Patient
b. Carrier
c. Reservoir
d. Infectious

Answers
1. a **2.** d **3.** b **4.** b **5.** b

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CHAPTER 28

Antimicrobial Stewardship

CHAPTER PREVIEW

- Implementation of Antimicrobial Stewardship Program
- Monitoring the Compliance to Antimicrobial Stewardship Program
- Rational Use of Antimicrobial Agents
- Hospital Antibiogram

INTRODUCTION

Antimicrobial stewardship program (AMSP) provides strategies for rationalizing the use of antimicrobials in the hospital.

Definition

The Centers for Disease Control and Prevention (CDC) has defined antimicrobial stewardship as use of the right antimicrobial agent, for the right patient, at the right time, with the right dose, route and frequency, causing the least harm to the patient and future patients.

Why AMSP is Needed?

Antimicrobial stewardship program in a hospital is required for the following reasons.

- **The rising threat of antimicrobial resistance (AMR):** Extensive use of antimicrobials is the single most important factor for the bacteria to undergo mutation, which leads bacteria to become resistant to antimicrobials and then the resistant strain flourishes exponentially in the presence of selective pressure of antimicrobials.
- **Misuse and over-use of antimicrobials** such as use without a prescription, overuse for self-limiting infections, non-bacterial infections, or treatment of colonizer/contaminant.
- **Widespread use of antimicrobials in other sectors** such as animal, agriculture, etc.
- **Limited availability of newer drugs** because of poor antimicrobial research, as there is a high-risk of developing resistance to the newer drugs.

IMPLEMENTATION OF ANTIMICROBIAL STEWARDSHIP PROGRAM

The key steps of implementation of AMSP in a hospital are as follows.

Administrative Support (Leadership)

The most important prerequisite for implementing AMSP is a strong administrative support. They should be publicly committed to the program and provide necessary funding and infrastructure support.

Formulating AMS Team

Antimicrobial stewardship team (AMS team) is a multidisciplinary committee that is responsible for framing, implementing, and monitoring the compliance with the antimicrobial policy of the hospital. Members of the AMS team include an infectious disease physician, a microbiologist, clinical pharmacists, an infection control officer, and an officer-in-charge of pharmacy.

Infrastructure Support

Infrastructure support is essential to initiate appropriate pathogen-directed antimicrobial agent at the earliest.

- **Support from the microbiology laboratory**
  - *Automations:* Facility for automated culture (e.g. BacT/ALERT), identification (MALDI-TOF) and sensitivity (e.g. VITEK) should be available.
  - *Biomarkers* such as procalcitonin and C-reactive protein (CRP) must be available (discussed subsequently).
  - *Molecular tests:* Facility to perform rapid molecular tests must be available; e.g. Biofire FilmArray multiplex PCR.
  - *Emergency laboratory:* Emergency lab functioning round the clock is a marker of a quality microbiology laboratory.
- **Hospital information system (HIS):** A Fully functional HIS, including a laboratory information system, will augment the stewardship program by many folds.
- Supporting manpower availability.

Framing Antimicrobial Policy

Every hospital should frame their own hospital antimicrobial policy which is usually a pocket handbook, comprising of

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system/syndrome wise indications for antimicrobial choice and their dosage.

- ❖ It should be prepared by AMS team after discussion with all the clinicians, microbiologists and administrators.
- ❖ The policy must be compliant with the standard national and international antimicrobial guidelines and local antibiogram pattern.
- ❖ Common consensus among all clinicians must be reached, before framing the policy; which facilitates better adherence to policy.

Implementing AMS Strategies

Two types of strategies are available for implementing AMSP.

1. **Front-end strategies (formulary restriction):** These refer to limited dispensing of restricted antimicrobials (e.g. colistin) by the hospital pharmacy, procurement of which requires prior authorization by the AMS team.
   - This strategy is difficult to implement because it directly compromises the clinician's freedom to choose antimicrobials.
   - More so availability of the AMS team to give approval all the time is a challenge, especially in emergency situations.
2. **Back-end strategies (prospective audit and feedback):** It involves daily rounds conducted by the AMS team, which discusses cases with the clinical team and provides advice on the rational use of antimicrobial therapy. This strategy is more easily accepted by clinicians as it promotes constructive discussion between AMS and clinical teams.

Education and Training

Similar to any other healthcare program, AMSP also needs continuous education, training, motivation and assessment of healthcare providers. Developing antimicrobial stewardship requires behavioral change in individuals. Hence, adequate motivational education is a must to bring in such change.

MONITORING THE COMPLIANCE TO ANTIMICROBIAL STEWARDSHIP PROGRAM

It is said that "if you cannot measure it, you cannot improve it". Measurement of the compliance to AMSP is achieved by looking at both process and outcome indicators.

1. **Policy adherence indicator** (process indicator): This is achieved by conducting antimicrobial stewardship audit as described under backward strategy. Both prescription and administrative compliance can be calculated.

Indicators of Prescription Compliance

Examples of prescription compliance indicators include:

- ❑ The percentage of time the empirical antibiotic is given is according to the suspected infectious syndrome.
- ❑ Percentage of time the empirical antibiotic is modified according to antimicrobial susceptibility report.
- ❑ Percentage of time cultures are taken before the start of antibiotics.
- ❑ Percentage of time the choice of surgical antimicrobial prophylaxis given is according to the policy.

Contd...

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Indicators of Administrative Compliance

Examples of administrative compliance indicators include:

- ❑ Percentage of time the antibiotic is administered correctly dose, correct frequency, correct route (IV, oral or infusion).
- ❑ Percentage of time the surgical antimicrobial prophylaxis is administrated at the correct dose, correct time and correct frequency.

2. **Antimicrobial usage outcome indicators** such as defined daily dosage (DDD) and days of therapy (DOT). These indicators are used to estimate the antibiotic consumption.
3. **AMR outcome indicator**: The change in AMR pattern is analyzed by conducting periodic AMR surveillance.
4. **Clinical outcome indicators** such as morbidity (e.g. length of stay) and mortality (e.g. infection-related deaths) indicators.
5. **Financial outcome indicators** such as antimicrobial cost per patient day or per year or per admission.

RATIONAL USE OF ANTIMICROBIAL AGENTS

When prescribing antimicrobial agents, clinicians should consider the following advice.

Prescribe Only when Indicated

Prescribe antibiotics only when it is indicated. There are various conditions where antibiotics are not required.

- ❖ **Diarrhea:** Oral rehydration solution is the mainstay of treatment, not antibiotics. More so, the most common cause of diarrhea is viral etiology.
- ❖ **Upper respiratory tract infections** such as common cold and sore throat, where the primary cause is viral infections (except when bacterial infections such as streptococcal sore throat or diphtheria are strongly suspected).
- ❖ When an **alternative diagnosis** is suspected/confirmed such as dengue, chikungunya, malaria, etc.
- ❖ **Prophylaxis:** Routine antibiotic prophylaxis should not be given to prevent infection, except for particular situations such as cotrimoxazole prophylaxis in HIV-infected individuals.

Culture of Cultures

Antibiotics should always be started only after site-specific specimens are collected for culture. If specimens are collected after antibiotic start, then cultures become false-negative and thus it will not help in targeted therapy.

Empirical vs Targeted Therapy

Empirical therapy: Empirical antibiotics should not be given randomly, but based on three important elements.

- ❖ The infective syndrome is likely to be present.
- ❖ The common etiological bacterial agents for that infective syndrome.
- ❖ The local antibiogram for those organisms, indicating the antimicrobial resistance pattern.

Targeted or pathogen-directed therapy: The empirical therapy should be modified subsequently, based on anti-

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microbial susceptibility test (AST) report. The modifications may be of two types—escalation or de-escalation.

Escalation vs De-escalation Approach

There are two approaches by which antimicrobial agents are prescribed—escalation and de-escalation.

- The approach needs to be chosen based on local antimicrobial resistance pattern and the spectrum of activity of the antibiotic.
- Antibiotics prescribed for an organism can be ranked based on their spectrum of activity and local antimicrobial resistance pattern.
- For example; in hospital X, the antibiotics given for gram-negative organisms such as *E. coli* are ranked according to decreasing order of susceptibility: colistin (rank-1) → tigecycline → carbapenems → piperacillin-tazobactam → cefoperazone sulbactam → amikacin → cefepime → ceftazidime → cotrimoxazole → ciprofloxacin → ceftriaxone (lowest rank).

Escalation Approach

This approach is chosen if the local antimicrobial resistance pattern is unlikely and/or the patient is clinically stable. The empirical therapy is started with a narrow-spectrum antibiotic (e.g. ceftriaxone for *E. coli*). If AST report shows resistance, then can be escalated to a higher rank antibiotic subsequently (e.g. meropenem for *E. coli*).

De-escalation Approach

This approach is chosen if the local antimicrobial resistance pattern is expected to be high and/or patient is critically ill. Empirical therapy is started with broad-spectrum antibiotics (e.g. meropenem for *E. coli*). If AST report shows susceptible, it can be de-escalated to a narrow-spectrum antibiotic subsequently (e.g. ceftriaxone for *E. coli)*.

However, the reserved/restricted antimicrobials such as colistin and tigecycline should not be given as empirical therapy even under de-escalation strategy. They should be prescribed only when the AST report is available and shows resistance to all other tested antimicrobials.

Site-specific Antimicrobials

Only those antimicrobials should be prescribed that are active at the infection site. The following antimicrobials are not active at the respective sites and therefore should be excluded from treatment.

- **Lungs:** Daptomycin is not active at the respiratory site as it gets inactivated by pulmonary surfactant.
- **CSF:** Most oral antibiotics, 1st, and second-generation cephalosporins, tetracyclines, macrolides, quinolones and clindamycin are not active in CSF.
- **Urine:** Antibiotics such as chloramphenicol, macrolide and clindamycin should be avoided in UTI; as they do not achieve adequate urinary concentrations.

Avoid Administration Errors

Antimicrobials must be administered at the correct dose (as per the age/body weight), and correct frequency and duration of therapy.

- **Loading dose:** Certain concentration dependent antimicrobials such as aminoglycoside, vancomycin and colistin should be administered with a loading dose.
- **Infusion:** The efficacy of certain antimicrobials such as vancomycin is better when mixed with saline and given as an IV infusion over 2–3 hours.
- **Renal adjustment:** The dosage of nephrotoxic drugs (such as aminoglycoside, vancomycin, and colistin) should be adjusted according to the creatinine clearance.

MIC-guided Therapy

The AST can be performed by disk diffusion or by MIC (minimum inhibitory concentration)–based method; the latter being more accurate and reliable. There are certain situations, where the antibiotic treatment is MIC-guided.

- Clinical conditions such as endocarditis, pneumococcal meningitis/pneumonia, etc.
- MIC helps to select the most appropriate antibiotic among the susceptible antibiotics.
- Vancomycin for *S. aureus*: Vancomycin should be avoided for treatment if MIC is >1 µg/mL.

Timely Stoppage of Antimicrobial

Antimicrobial agent must be stopped at appropriate time, which may be determined by clinical improvement or after obtaining negative culture or by use of biomarkers.

Biomarkers-guided Therapy

Biomarkers such as procalcitonin (PCT) or C-reactive protein (CRP) may be used for predicting bacterial infection. PCT is more reliable marker than CRP.

Procalcitonin (PCT)

It is a peptide precursor of the hormone calcitonin, secreted at very low level (<0.05 ng/mL) normally by the body, but the level goes up by manifolds in bacterial infection. The various diagnostic application of PCT include:

- To differentiate bacterial vs viral infection.
- For predicting bacterial infections such as local or systemic infection (e.g. bacterial sepsis, or bacterial pneumonia), depending upon PCT level and associated clinical presentation.
  - 0.05–2.0 ng/mL indicates possible local infection
  - 2–10 ng/mL indicates systemic infection
  - >10 ng/mL indicates septic shock.
- Helps to decide whether to continue antibiotics in culture-negative cases.
- Helps to decide whether to stop antibiotics.
- Severity: PCT levels parallel with the severity of the bacterial infections; higher the PCT level, severe is the infection. Therefore PCT can be used as prognostic indicator.

However, PCT should not be used alone, but should always be used in adjunct with cultures.

Misuse of Antimicrobials

The following are the common examples of misuse of antimicrobials that should be avoided.

- **Avoid overlapping spectra:** Meropenem and piperacillin–tazobactam combination therapy for double gram-negative

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coverage should be avoided as both these drugs belong to the beta-lactam group of antimicrobials and therefore share similar antibacterial spectra.

- ❖ **Redundant antibiotic:** Meropenem and metronidazole combination therapy for suspected gram-negative/anaerobic sepsis should be avoided as meropenem is active against anaerobes in addition to gram-negative bacteria. Therefore, metronidazole can be withdrawn from therapy.
- ❖ **Ineffective antibiotic:** Cloxacillin in MRSA (methicillin resistant *S. aureus*) infection is ineffective and therefore should be avoided. Vancomycin is the drug of choice for MRSA.
- ❖ **Inferior antibiotic:** Vancomycin is an inferior cell wall acting agent compared to cloxacillin for MSSA (methicillin susceptible *S. aureus*) infection.

HOSPITAL ANTIBIOGRAM

An **antibiogram** is an overall profile of antimicrobial susceptibility testing results of a specific microorganism to a battery of antimicrobial agents **(Table 28.1)**. It is the responsibility of the department of Microbiology to construct a hospital antibiogram and share it with clinicians. It has the following uses.

Table 28.1: Hospital antibiogram of gram-negative bacteria for the year 2020, expressed in terms of susceptibility rate.

	Ciprofloxacin	Amikacin	Ceftazidime	Piperacillin-tazobactam	Meropenem	Colistin
E. coli	25	85	55	75	82	99
Klebsiella	15	75	35	65	70	85
Pseudomonas	32	80	60	80	80	99
Acinetobacter	29	70	10	18	20	99

- ❖ Antibiogram guides the clinicians in selecting the best empirical antimicrobial treatment in the event of pending culture and susceptibility results.
- ❖ It is also a useful tool for detecting and monitoring trends in antimicrobial resistance within the hospital.
- ❖ Antibiograms can also be used to compare susceptibility rates across institutions and track resistance trends, thereby contributing to the national AMR surveillance database.

EXPECTED QUESTIONS

I. Write short notes on:
1. Strategies of antimicrobial stewardship program.
2. Monitoring of antimicrobial stewardship program.

II. Multiple choice questions (MCQs):

1. Antimicrobial stewardship program in a hospital is required for the following reasons, *except*:
a. Rapid development of antimicrobial resistance
b. Misuse and over-use of antimicrobials
c. Widespread use of antimicrobials in humans compared to animal industry
d. Poor antimicrobial research

2. Who can act as an antimicrobial steward?
a. Infectious disease physician
b. Clinical microbiologists
c. Medicine consultant
d. Any of the above

3. Which of the following is not a back-end strategy of antimicrobial stewardship?
a. Prospective audit and feedback is an example
b. Formulary restriction is an example
c. It is labor intensive than front-end strategy
d. Sustainable than front-end strategy

4. Maximum antibiotic consumption occurs in:
a. Human therapeutic use
b. Human non-therapeutic use
c. Animal therapeutic use
d. Animal non-therapeutic use

5. In which of the following infectious disease, antibiotics are required?
a. Diarrhea
b. Influenza
c. Malaria
d. None of the above

6. The following are the uses of antibiogram, *except*:
a. It guides the clinicians in selecting the best empirical antimicrobial treatment in the event of pending culture and susceptibility results
b. It is an useful tool for detecting and monitoring trends in antimicrobial resistance within the hospital
c. It can be used to compare susceptibility rates across institutions and track resistance trends
d. Useful to guide the clinicians in selecting the best targeted antibiotic therapy

Answers
1. c **2.** d **3.** b **4.** d **5.** d **6.** d

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