CHAPTER-7

ANTIMICROBIAL ACTIVITY
7.1 Antimicrobial activity

The mepacrine, antimalerials and pamaquin were shaped from dyes & in 1935 dye (Prontosil) joined sulphonamides was first agreed as a product of systematic analysis by Domagk. The data obtained from sulphonamides in meningitis, puerperal sepsis & pneumonia were dramatic and occurred a upheaval in medical & scientific assumed.

Classification of Antimicrobial Drugs:

On the basis of type of organism antimicrobial agents can be divided as follow:

- Antifungal drugs
- Antibacterial drugs
- Anthelmintic drugs
- Antiviral drugs
- Antiprotozoal drugs

![Antifungals](image-url)
Antifungal Drugs

• Also called antimycotic drugs
• Used to treat two types of fungal infection:
  – Superficial fungal infections
    • (skin or mucous membrane)
  – Systemic fungal infections
    • (lungs or central nervous system)
Antifungal agents

Amphotericin B

Flucytosine (5-Fluorocytosine)

Miconazole

Ketoconazole

Itraconazole

Fluconazole
MECHANISMS OF ACTION OF ANTIBACTERIAL DRUGS

Mechanism of action include:
- Inhibition of cell wall synthesis
- Inhibition of protein synthesis
- Inhibition of nucleic acid synthesis
- Inhibition of metabolic pathways
- Interference with cell membrane integrity

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<tr>
<th>Antibiotic class</th>
<th>Parent scaffold</th>
<th>Derivatized scaffolds</th>
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<tbody>
<tr>
<td>Fluoroquinolones</td>
<td>Nalidixic acid</td>
<td>Gatifloxacin, Moxifloxacin</td>
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<tr>
<td>Nitroimidazoles</td>
<td>Metronidazole</td>
<td>PA-824, OPC-67683</td>
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<td>Oxazolidinones</td>
<td>Linezolid</td>
<td>PNU-100480</td>
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<tr>
<td>1,2-ethylene diamine</td>
<td>Ethambutol</td>
<td>SQ109</td>
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Antibacterial agents
Minute antimicrobials have useful activity across various of these groups. Some examples are metronidazole inhibits necessitate anaerobic bacteria (like Clostridium perfringens) and few protozoa that rely on anaerobic pathways (like Trichomonas vaginalis).

Antimicrobial medicine mainly of two type as follow:

- Bacteriostatic: Drug which can inhibit the bacteria. For example chloramphenicol and sulphonamides, tetracyclines.
- Bactericidal, Drug which can kill the bacteria. For example rifampicin, cephalosporins, penicillins, isoniazide and aminoglycosides.

**Classification of Organisms:**

Staphylococcus aureus is species of schizomycetes class; having Eubacterials order, micrococeaceac family and staphylococcus genus.
Escherichia coli is species of schizomycetes class; having Eubacterial order, Enterobacteriaceae family and Escherichia genus.

Bacillus subtilis is species of schizomycetes class; having Eubacterials order, Bacteriodaceae family and fusobacterium streptobacillus and sphaerophorus genus.

Pseudomonas aeruginosa is species of schizomycetes class; having pseudominodales order, pseudominadaceae family and pseudomonas genus.

**Identification Techniques of the Organisms:**

The organisms were identified by using the following strains [239, 240].

- Schiff technique periodic acid
- Gram strains
- Zeil Nelsonm acid fast strains

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The organisms were identified by using the following strains.

- Schiff technique periodic acid
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**Measurement Methods:**

The below situation must be applied to evaluate antimicrobial activity.
• There should be an cherished contact between substance to be analyzed and test organisms.

• Necessary situation should be applied to the growth of microorganisms.

• Situation should be similar throughout the experiment.

• Aseptic/sterile surrounding should be regulated.

Different techniques have been applied from period to period by various workers to measure the antimicrobial activity. The measurements can be made by the below techniques [241-243].

An antimicrobial is a material that destroys or constrains the growth of microorganisms such as fungi, protozoans or bacteria. Antimicrobial agents may either destroy microbes or inhibits the growth of microbes (microbiostatic). Disinfectants are antimicrobial materials utility on non-quick things or outward of body.

The antiquity of antimicrobials creates with the interpretations of Joubert and Pasteur, who have developed that one kind of bacteria could inhibits the progress of alternative. They did not identify at that time that the reason one bacterium abortive to cultivate was that the additional bacterium was generating an antibiotic. Theoretically, antibiotics are only those materials that are generated by one microbe that destroyed, or inhibits the progress, of other microbes. Of course, in today’s shared tradition, the period antibiotic is applied to refer almost any drug that efforts to rid your form of a bacterial infected. Antimicrobials covers not fair antibiotics, but unnaturally fashioned mixtures as well.

The developments of antimicrobials such as tetracycline and penicillin covered the path for well health for lots around world. Before penicillin became a practicable medical management in the timely 1940s, no true medication for gonorrhea and pneumonia excited. Patients with diseased abrasions regularly had to have injured limb eliminated, or face death from infection. Now, most of these infections can be treat easily with a small course of antimicrobials. However, with the discovery of microorganisms, antimicrobials have modified
and developed resistant to preceding antimicrobial agents. The old antimicrobial methodology was founded either on contagions or substantial metals, which may not have destroyed the microbe copiously, authorizing the microbe to remain, modification, and converted resistant to the poisons & heavy metals.

Antimicrobial nanotechnology is a fresh accumulation to the fight versus disease producing organisms, changing heavy metals & toxins may some day be a worthwhile different.

7.2 Bacteria

Bacteria are often slandered as the grounds of animal and human. However, certain bacteria, the actinomycetes yield antibiotics such as nocardicin and streptomycin; others live symbiotically in the guts of faunae (including humans) or away in their forms, or on the origins
of convinced plants, transforming N into a serviceable procedure. Bacteria put the tang in yogurt & the sour in sourdough bread; microorganisms help to discontinuity down dead organic substance; bacteria made up base of the nutrition web in many atmospheres. Bacteria are of such enormous significance because of their punishing flexibility, capacity for quick progress & replica, and prodigious age - the oldest vestiges known, approximately 3.5 billion years old, are remains of bacteria-type organisms.

Bacteria are microbes such fomed by just one cell. They are proficient of growing by themselves, as they have the capacity to distribute. Their figures diverge, & doctors use these properties to detach them into groups. The bacteria (singular: bacterium) were group of single cellular microorganisms. They are little tiny, most being roughly 0.5 to 2.0 µm in diameter. Most of such organisms have a comparatively simple morphology & cellular organization. Bacteria are omnipresent in every territory on Earth, progress in soil, radioactive waste, acidic hot springs, seawater, & deep in the Earth's crust. There are especially 40 million bacterial cells in a gram of soil sample & fresh water contain a million bacterial cells in a milliliter; in all, there are roughly five nonillion \(5 \times 10^{30}\) bacteria on Earth, producing much of the world's biomass. Bacteria are crucial in reprocessing nutrients, & Large significant steps in nutrient cycles be contingent on microorganisms, such as the obsession of N from the surrounding. However, most of such bacteria have not been categorized & only nearby half of the phyla of bacteria have sample that can be refined in the laboratory.

Bacteria may be detected on top of elevations, the bottom of sincere oceans, in the guts of animal, in the ice-covered rocks, and ice of Antarctica. One eye that has allowed them to banquet so far & long is their aptitude to go latent for protracted period. The cell walls of bacteria are made out of peptidoglycan while that of fungi and plants are made of chitin and cellulose respectively. The cell wall is used in characterizing bacteria into groups. Bacteria have been grouped in two groups. Gram-positive of cell wall having thick peptidoglycan or murein layer and teichoic acid; while Gram-negative of cell wall having thin peptidoglycan and lipopolysaccharide-containing membrane.
In the present study, bacterial and fungi strains such as Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger, Aspergillus clavatus for antimicrobial activity and M. tuberculosis were used as test bio-organism for the antituberculosis activity testing. Accordingly their general information about their history and living style are briefly discussed.

7.3 Staphylococcus aureus
Staphylococcus aureus is a microbial species termed produced Greek meaning the "golden grape-cluster berry". Also recognized as "golden staph" & Oro staphira, it is a facultative anaerobic Gram +ve coccal bacterium. It is regularly originate as part of the ordinary skin flora on skin & nasal passag. It is projected 20% of the human crowd are extended-term transporters of S. aureus is the greatest collective classes of staphylococcus to reason Staph infections. S. aureus is a usefull pathogen due to a amalgamation of bacterial immuno-evasive approaches. One of these approaches is the generation of carotenoid staphyloxanthin pigment, which is accountable for the properties of golden colour of S. aureus colonies. This dyes acts as a virupenicillin lence, primarily by act as a antioxidant bacterial which usefull the microbe evade the reactive oxygen species which the crowd immune system utilized to destroyed pathogens. S. aureus may occur as a commensal on skin; it also follows in the nose regularly.
Staphylococcus aureus
Escherichia coli generally shortened *E. coli* is a Gram-ve, rod-shaped bacterium that is generally detected in the minor intestine of warm-blooded creatures (endotherms). Most *E. coli* strains are meaningless, but some serotypes can creates thoughtful food killing in humans, & are infrequently accountable for merchandise recalls due to food adulteration. The inoffensive strains are part of the regular flora of the instinctive, and can profit their swarms by creating vitamin K₂, & by stopping the formation of pathogenic microorganisms within the intestine. *E. coli* & related microbes organize about 0.1% of fecal-oral and gut flora transmission is the major way through which pathogenic strains of the bacterium foundation disease. Cells are efficient to continue external of body for imperfect amount of time, which made them perfect indicator organisms to examination environmental samples for fecal pollution. There are, however a
progressig body of investigation that has scrutinized environmentally tenacious *E. coli* which can endure for prolonged phases of time external of the host.
7.5 Bacillus megaterium

*Bacillus megaterium* is a virgate, Gram +ve, endospore producing, aerotolerant sample of bacteria used as a soil inoculant in farming and gardening. Micobes are arranged in the streptobacillus form. *Bacillus megaterium* are rod fashioned bacterium & one of the chief eubacteria detected in soil. Collections of the bacteria were often detected in manacles where the lockups are combined together by polysaccharides on the cell walls. *Bacillus megaterium* is able to continue in some exciting situation like as desert environments because of spores creates. There are comfortable situation the bacteria can persist. Sometimes these particular microbe can be detected on general area that are often affected. *Bacillus megaterium* yields penicillin amidase applied for preparing penicillin. It creates enzymes for adapting corticosteroids, as well as various amino acid dehydrogenases.

*Bacillus megaterium*
7.6 Proteus vulgaris

*Proteus vulgaris* is a rod-like structure, gram-ve bacterium which restricts the intestinal tracts of animals and humans. It could be detected in soil, water & fecal matter. It is clustered in enterobacteriaceae & is cunning pathogen of humans. It is called to cause urinary tract contagions & wound contagions. The word *Proteus* means changeability of procedure, as incarnate in the Homeric verses in Proteus, "the old species of sea," who inclines the sealflocks of Poseidon & has the talent of boundless alteration. The first use of the word “Proteus” in microbiological terminology was make by Hauser in 1850. Who explain below this period 3 kinds of creatures which he inaccessible from decomposed essence. One of the 3 kinds Hauser recognized was *Proteus vulgaris* so this creature has extended antiquity in Microbiology. Over the historical 2 eras the genus *Proteus*, & in specific *P. vulgaris*, has endured a several of major taxonomic modifications. In 1982, *P. vulgaris* was detached into 3 biogroups on the beginning of indole manufacture. Biogroup unique was indole undesirable and shows a new classes: *P. penneri*; while biogroup 2 & 3 persisted together as *P. vulgaris*. 
Figures of *Proteus vulgaris*

**Measurement Methods:**

The below situation must be applied to evaluate antimicrobial activity.

- There should be an cherished contact between substance to be analyzed and test organisms.

- Necessary situation should be applied to the growth of microorganisms.
7.7 EXPERIMENTAL

The antibacterial potency of the substances was evaluated by disc plate procedure. The test discs were having 50 microgram per disc of the sample complex. The action was exposed against gram +ve bacteria are *Bacillus megaterium* [MTCC (121)], *Staphylococcus aureus* [MTCC(96)], and gram –ve bacteria *Proteus vulgaris* [MTCC(1771)], *Escherichia coli* [MTCC(443)].

Preparation of Media:

For bacterial activity nutrient agar is used. Nutrient agar is prepared as follows:

1) Meat Extract : 3 gm
2) Peptone : 5 gm
3) Agar Agar : 15 gm
4) Sodium chloride : 5 gm

All the above ingredients were mixed in one liter distilled water and heated to dissolve all the ingredients. The medium was stabilized in autoclave at 15 pound pressure at 125°C for 20 minutes. The medium was cooled down to 45°C and 20 ml poured in sterilized Petri-dish. The pH of the medium was adjusted between 7.0 to 7.5

The culture of the above organism was prepared in nutrient broth dissolved in distilled water. The content of nutrient broth are:

1) Beef extract : 10 gm
2) Peptone : 10 gm
3) Sodium chloride : 5 gm

After sterilizing the above media, it was used for the culture purpose. The culture was ground at 37°C in incubator. With the help of swab the culture was spread over the agar plates, under specific condition

5 mm diameter paper discs were prepared and were sterilized in autoclave. The solution of the test compound was kept over these paper discs with the help of micropipette. These discs
were dried to remove the solvent. Sterile test compound coated by discs were kept in Petri dish containing culture media. The discus was pressed to sterile on media and Petri dishes were incubated for 24 hours at 37°C. After the incubations the zone of inhibition was measured.

![Figure 7.1 Antibacterial Activities of COMPOUND A1-A19](chart)

A short review of results of antibacterial screening of the compounds of this section is mentioned here:

(I) **Against Staphylococcus aureus:**
Maximum activity were found in compounds (A18, A19) zone of inhibition-13.0 m.m. and minimum activity were found in compounds (A7, A11) zone of inhibition -6.0 m.m.

(II) **Against Bacillus megaterium:**
Maximum activity were found in compounds (A13, A17) zone of inhibition -14.0 m.m where as minimum activity were found in compound (A5) zone of inhibition -5.0 m.m.
(III) **Against Escherichia coli:**
Maximum activity were found in compounds (A3, A18, A19) zone of inhibition -12.0 m.m and minimum activity were found in compounds (A4) zone of inhibition -3.0 m.m

(IV) **Against Proteus vulgaris:**
Maximum activity were found in compound (A9, A3, A18, A19) zone of inhibition -16.0 m.m (near to standard drug) and minimum activity were found in compounds (A4) zone of inhibition -4.0 m.m

![Figure 7.2 Antibacterial Activities of COMPOUND B1-B19](image)

A short review of results of antibacterial screening of the compounds of this section is mentioned here:

(I) **Against Staphylococcus aureus:**
Maximum activity were found in compounds (B18, B19) zone of inhibition-13.0 m.m. and minimum activity were found in compounds (B7, B11) zone of inhibition -6.0 m.m
(II) **Against Bacillus megaterium:**
Maximum activity were found in compounds (B13, B17) zone of inhibition -14.0 m.m where as minimum activity were found in compound (B5) zone of inhibition -5.0 m.m.

(III) **Against Escherichia coli:**
Maximum activity were found in compounds (B3, B18, B19) zone of inhibition -12.0 m.m and minimum activity were found in compounds (B4) zone of inhibition -3.0 m.m

(IV) **Against Proteus vulgaris:**
Maximum activity were found in compound (B9, B3, B18, B19) zone of inhibition -16.0 m.m (near to standard drug) and minimum activity were found in compounds (B4) zone of inhibition -4.0 m.m

**Figure 7.3 Antibacterial Activities of COMPOUND C1-C19**

A short review of results of antibacterial screening of the compounds of this section is mentioned here:
(I) **Against *Staphylococcus aureus***:

Maximum activity were found in compounds (C18, C19) zone of inhibition-13.0 m.m and minimum activity were found in compounds (C7, C11) zone of inhibition -6.0 m.m.

(II) **Against *Bacillus megaterium***:

Maximum activity were found in compounds (C13, C17) zone of inhibition -14.0 m.m where as minimum activity were found in compound (C5) zone of inhibition -5.0 m.m.

(III) **Against *Escherichia coli***:

Maximum activity were found in compounds (C3, C18, C19) zone of inhibition -12.0 m.m and minimum activity were found in compounds (C4) zone of inhibition -3.0 m.m

(IV) **Against *Proteus vulgaris***:

Maximum activity were found in compound (C9, C3, C18, C19) zone of inhibition -16.0 m.m (near to standard drug) and minimum activity were found in compounds (C4) zone of inhibition -4.0 m.m

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**Figure 7.4 Antibacterial Activities of **COMPOUND D1-D19**

<table>
<thead>
<tr>
<th>Antibacterial activities</th>
<th>S.aureus (+Ve)</th>
<th>B.megaterium (+Ve)</th>
<th>E.coli (-Ve)</th>
<th>P.vulgaris (-Ve)</th>
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<tbody>
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<td>D1</td>
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<td>D19</td>
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Ampicillin

Gentamicin

Antibacterial activities
A short review of results of antibacterial screening of the compounds of this section is mentioned here:

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Maximum activity were found in compounds (D18, D19) zone of inhibition-13.0 m.m. and minimum activity were found in compounds (D7, D11) zone of inhibition -6.0 m.m

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Maximum activity were found in compounds (D13, D17) zone of inhibition -14.0 m.m where as minimum activity were found in compound (D5) zone of inhibition -5.0 m.m.

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Maximum activity were found in compounds (D3, D18, D19) zone of inhibition -12.0 m.m and minimum activity were found in compounds (D4) zone of inhibition -3.0 m.m

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Maximum activity were found in compound (D9, D3, D18, D19) zone of inhibition -16.0 m.m (near to standard drug) and minimum activity were found in compounds (D4) zone of inhibition -4.0 m.m