2.1 Antibiotics:

The Antibiotics or antibacterials are used in the treatment of bacterial infection; they may kill or inhibit the growth of bacteria. Antibiotics are a type of antimicrobial. Several antibiotics are effective against fungi also, but are not effective against viruses such as common cold and influenza.

Science historian Howard Markel was actually coined the term “antibiotics” in 1860. But the term “antibiotic” had been used first time by Selman Waksman, the microbiologist who discovered streptomycin, in 1943 for medical sense.

Streptomycin is a bactericidal antibiotic and it was derived from the actinobacterium *Streptomyces griesus*. It is on the WHO’s List of *Essential medicines*. Adverse effect of this drug is *ototoxicity, nephrotoxicity, fetal auditory toxicity, and neuromuscular paralysis*.31-32

The antibiotics are of three types for the specific microbial coverage:
1. Methicillin-resistant Staphylococcus aureus (MRSA) 
2. Pseudomonas aeruginosa 
3. Vancomycin-resistant Enterococcus (VRE) 

Methicillin-resistant *Staphylococcus aureus* is a bacterium accountable for several difficult to cure contagions in humans. It is also called oxacillin-resistant *Staphylococcus aureus* (ORSA). MRSA is any strain of *Staphylococcus aureus* that has developed, through the process of natural selection, confrontation to β-lactam antibiotics, which include the Penicillins (methicillin, dicloxacillin, nafcillin, oxacillin, etc.) and the cephalosporins. Strains incompetent to fight back with these antibiotics are known as Methicillin-sensitive *Staphylococcus aureus* (MSSA). The development of such resistance does not cause the entity to be more essentially lethal than strains of *S. aureus* which have no antibiotic confrontation, but confrontation does make MRSA infection more challenging to treat with standard types of antibiotics and therefore more risky.

Meticillin was developed by Beecham in 1959. It was previously used to treat infections caused by susceptible Gram+ve bacteria, in particular, penicillinase producing bacteria such as *S. aureus* which would otherwise be resilient to most penicillin. Its role in treatment has been largely switched by flucloxacillin and dicloxacillin, but the term meticillin-resistant *S. aureus* continues to be used to describe *S. aureus* strains resistant to all Penicillins.
Pseudomonas aeruginosa is a common Gram-negative bacterium that can cause disease in plants and animals, together with humans. It is citrate, catalase, and oxidase +ve. It uses an extensive range of organic substantial for food; in animals, its versatility allows the entity to infect impaired tissues or those with bargain immunity. The signs of such taints are generalized inflammation and sepsis. If such colonizations take place in acute body organs, such as lungs, urinary tract, and kidneys, then consequences can be fatal\textsuperscript{36}. It is a Gram-negative, aerobic, bacillus bacterium with unipolar motility\textsuperscript{37}. An opportunistic human pathogen, \textit{P. aeruginosa} is also opportunistic pathogen in plants\textsuperscript{38}. \textit{P. aeruginosa} is the type species of the genus \textit{Pseudomonas} (Migula)\textsuperscript{39}.

Cefepime is a 4\textsuperscript{th} generation cephalosporin antibiotic. Cefepime has a comprehensive spectrum of activity against gram +ve and gram -ve strains of bacteria, with greater activity than third-generation agents. Cefepime was developed by Bristol-Myers Squibb\textsuperscript{40-41} and marketed beginning in 1994. Now it is available as a generic drug and sold underneath a range of trade names worldwide.
Vancomycin resistant *Enterococcus*, or vancomycin resistant enterococci (VRE), are bacterial strains of the genus *Enterococcus* that are resistant to the antibiotic vancomycin\(^42\). High-level vancomycin-resistant *E. faecalis* and *E. faecium* are isolates first in 1980's\(^43\).

*Enterococcus* shows 6 different kinds of vancomycin resistance: Van-A, Van-B, Van-C, Van-D, Van-E and Van-G\(^44\). Mechanism of resistance to vancomycin includes the modification in peptidoglycan synthesis pathway\(^45\). Linezolid an antibiotic used for the cure of serious contagious due to Gram-positive bacteria that are resilient to other antibiotics. Linezolid is active against maximum Gram-positive bacteria which cause disease, together with streptococci, vancomycin (VRE). Linezolid was discovered in 1990s by a team at Pharmacia and Upjohn Company and first permitted to use in 2000. It is on the World Health Organization's List of Essential Medicines, the most important medications needed in a basic health system\(^46\).

![Linezolid (INN)](image)

The oxazolidinones are protein synthesis inhibitors: they stop the growth and reproduction of bacteria by disrupting translation of messenger RNA (mRNA) into proteins in the ribosome. While its mode of action is not fully understood\(^47\), linezolid seems to work on first step of protein synthesis, *initiation*, unlike other protein synthesis inhibitors, that inhibit *elongation*\(^48\).
It does so by inhibiting the development of the *initiation complex*, composed of the 30S and 50S subunits of ribosome, t-RNA, and mRNA. Linezolid binds to the 23S portion of the 50S subunit (the center of peptidyl transferase activity)\(^49\), close to binding spots of chloramphenicol, lincomycin, and other antibiotics. Due to this exclusive mode of action, cross-resistance between linezolid and other protein synthesis inhibitors is highly infrequent or non-existent\(^{40, 50}\).

![Simplified schematic of m-RNA translation](image)

**Fig.-1:** Simplified schematic of m-RNA translation. Linezolid occupies the A site (at center) and prevents t-RNA from binding.

Linezolid is an absolutely synthetic drug: it does not found naturally (not like erythromycin and several other antibiotics) and was not industrialized by constructing upon a naturally occurring skeleton (not like most β-lactams, which are semisynthetic). Several methodologies and routes for synthesis of oxazolidinone, and synthesis of linezolid have been reported in the literature of chemistry\(^{51}\).

Tedizolid (earlier torezolid, trade name Sivextro)\(^{52}\) is an oxazolidinone-class antibiotic. Tedizolid phosphate is a prodrug of active compound tedizolid. It was established by Cubist Pharmaceuticals, following acquirement of Trius Therapeutics (inventor: Dong-A Pharmaceuticals), and is marketed to treat the acute
bacterial skin and skin structure contagions [known as complicated skin and skin-structure infections (cSSSIs)]

Tedizolid has been approved by the U.S. FDA on June 20, 2014, for the treatment of acute bacterial skin and skin structure infections (ABSSSI) caused by certain susceptible bacteria, together with *Staphylococcus aureus* (including methicillin-resistant strains, MRSA, and methicillin-susceptible strains), several *Streptococcus* species (*S. pyogenes*, *S. agalactiae*, and *S. anginosus* group including *S. anginosus*, *S. intermedius*, and *S. constellatus*), and *Enterococcus faecalis*.

Tedizolid is a second-generation oxazolidinone derivative that is 4-to-16-fold more potent against staphylococci and enterococci compared to linezolid.

Tedizolid phosphate (TR-701) is a plasma activated prodrug of intestinal phosphatases to tedizolid (TR-700) following administration of the drug either orally or intravenously. Once activated, tedizolid applies its bacteriostatic microbial activity by inhibition of protein synthesis by binding to the 50S ribosomal subunit of the bacteria.

### 2.2 Classification of Antibiotics:

On the basis of class, antibiotics are of the following types:

#### 2.2.1 Aminoglycosides:

Aminoglycoside is a medicinal and bacteriologic category of traditional Gram-negative antibacterial therapeutic agents that inhibit protein synthesis and contain as a portion of the molecule an amino-
modified glycoside (sugar)\textsuperscript{58, 59}, the term can also refer more generally to any organic molecule that contains amino-sugar substructures. Aminoglycoside antibiotics display bactericidal activity against gram-negative aerobes and some anaerobic bacilli where resistance has not yet arisen, but generally not against Gram-positive and anaerobic Gram-negative bacteria\textsuperscript{60}.

2.2.2 Ansamycins: Ansamycins is a club of secondary metabolites which exhibit antimicrobial activity against many Gram-positive and some Gram-negative bacteria and contains various compounds, among which, streptovaricins and rifamycins\textsuperscript{61}. In addition, these compounds exhibit antiviral activity concerning bacteriophages and poxviruses.

2.2.3 Carbacephem: Carbacephems comprise of synthetic antibiotics, based on structure of cephalosporin, a cephem. Carbacephems are analogous to cephems, but with a carbon substituted by sulfur. It inhibits bacterial cell division by inhibiting synthesis of cell wall.

2.2.4 Carbapenems: Carbapenems are antibiotics used for the treatment of infections known or suspected to be caused by multidrug-resistant (MDR) bacteria. Their use is mainly in hospitalized people. The carbapenem ertapenem is in the numerous first-line agents endorsed by Infectious Disease Society of America for the empiric cure of community attained intra-abdominal contagions of mild-to-moderate rigorouss. A 2015 methodical review found minute evidence that would support credentials of a best antimicrobial treatment for complex urinary tract infections, but acknowledged 3 high excellence trials supporting high treatment rates through doripenem, counting in patients with levofoxacin-resistant \textit{E. coli} contagions\textsuperscript{62}. The imipenem and meropenem are endorsed by the American Thoracic Society and the Infectious Disease Society of America as one of several first-line therapy options.
for people with late-onset hospital-acquired or ventilator-associated pneumonia, especially when *Pseudomonas, Acinetobacter, or extended spectrum β-lactamase producing Enterobactericeace* are suspected pathogens. Combination rehabilitation, typically with an aminoglycoside, is recommended for *Psuedomonas* infections to avoid resistance development during treatment. Serious and seldom fatal allergic responses can arise in people cured with carbapenems. Seizures are a dose-regulating toxicity to imipenem and meropenem both.

### 2.2.5 Cephalosporins (First, Second, Third, Fourth and Fifth generation):

Cephalosporins are β-lactam antibiotics and derived from *Acremonium* fungus (known as Cephalosporium). Together with cephamycins, they establish a sub-group of β-lactam antibiotics named cephems. Cephalosporins were discovered in 1945 and were first sold in 1964. Cephalosporins are specified for prophylaxis and cure of infections produced by bacteria vulnerable to this specific form of antibiotic. 1st generation cephalosporins are active principally against Gram +ve bacteria, and succeeding generations have improved activity against Gram -ve bacteria (albeit frequently with bargain activity against Gram +ve organisms).

Cephalosporins are bactericidal and have same mode of action as former β-lactam antibiotics (such as Penicillins), but are not as much of susceptible to β-lactamases. Cephalosporins interrupt the synthesis of the bacterial cell wall by inhibition of peptidoglycan synthesis. The peptidoglycan layer is imperative for cell wall structural veracity. Final transpeptidation stage in synthesis of the peptidoglycan is assisted by transpeptidases identified as penicillin-binding proteins (PBPd). PBPd impasse to D-Ala-D-Ala at the end of muropeptides.
(precursors of peptidoglycan) to crosslink peptidoglycan. $\beta$-lactam antibiotics mimic D-Ala-D-Ala site, thus permanently preventing PBP crosslinking of peptidoglycan.

2.2.6 Glycopeptides: Glycopeptide antibiotics are a class of drugs of microbial origin that are composed of glycosylated cyclic or polycyclic non-ribosomal peptides. Significant glycopeptide antibiotics include the anti-infective antibiotics vancomycin, teicoplanin, telavancin, ramoplanin and decaplanin, and the antitumor antibiotic bleomycin. Vancomycin is used if infection with methicillin-resistant Staphylococcus aureus (MRSA) is suspected.

2.2.7 Lincosamides (Bs): The first lincosamide discovered is named lincomycin, isolated from Streptomyces lincolnensis in a soil model from Lincoln, Nebraska. The usage of lincomycin has been mostly outdated by clindamycin which shows better antibacterial activity. Clindamycin also shows some activity against parasitic protozoa and used for toxoplasmosis and malaria. Lincosamides are normally used to treat Staphylococcus and Streptococcus and have proved useful in treating Bacteroides fragilis and some other anaerobes. They are used in the treatment of toxic shock syndrome and thought to directly block the M protein synthesis that leads to severe inflammatory response. Lincosamide antibiotics are one of the classes of antibiotics most associated with pseudomembranous colitis caused by Clostridium difficile.

2.2.8 Lipopeptides: A lipopeptide is a molecule consisting of a lipid connected to a peptide. Bacteria express these molecules. They are bound by TLR 2, which form an eterodimer with TLR6 or TLR1. Firm lipopeptides are used as antibiotics. Certain lipopeptides could have strong anifungtal and hemolytic activities. It has been validated that their activity is generally linked to interactions with the plasma
membrane and sterol components of plasma membrane could play a major role and can be their privileged partner of interaction.

2.2.9 Macrolides (Bs): The macrolides are consist of a large macrocyclic lactone ring to which one or more deoxy sugars, usually cladinose and desosamine, may be attached. The lactone rings are generally 14-, 15-, or 16-membered. Some macrolides have antimicrobial activity and are used as medicinal drugs. Macrolides are protein production inhibitors. The mechanism of action of macrolides is inhibition of bacterial protein biosynthesis, and they are supposed to do this by inhibiting peptidyl transferase from adding the growing peptide attached to tRNA to the next amino acid (similarly to chloramphenicol) as well as inhibiting ribosomal translation. Another potential mechanism is premature dissociation of the peptidyl-tRNA from the ribosome.

A 2008 *British Medical Journal* editorial highlights that combination of few macrolides and statins (used to lower cholesterol level) is not suitable and can lead to devastating myopathy. This is for the reason that some macrolides (clarithromycin and erythromycin, not azithromycin) are strong inhibitors of cytochrome P450 system, particularly of CYP3A4. Macrolides, primarily erythromycin and clarithromycin, similarly have a class result of QT prolongation that can lead to torsades de pointes.

2.2.10 Monobactams: Monobactams (e.g.: tigemonam, nocardicin A, and tabtoxin) are β-lactam compounds wherein the β-lactam ring is not fused to another ring (in compare to maximum other β-lactams, which have minimum two rings). They are active only against aerobic Gram-ve bacteria.
(e.g., Neisseria, Pseudomonas). Adversarial effects to monobactams can comprise skin rash and random unusual liver functions.

### 2.2.11 Nitrofurans:
Nitrofurans are a class of drugs typically used as antibiotics or antimicrobials. The defining structural component is a furan ring with a nitro group.

![Nitrofurazone](image)

### 2.2.12 Oxazolidinones:
Oxazolidinones are a class of compounds containing 2-oxazolidone in the structure. Oxazolidinones are mostly used as antimicrobials. The antibacterial effect of oxazolidinones is by working as protein synthesis inhibitors, targeting an initial step concerning the binding of N-formylmethionyl-tRNA to ribosome\(^ {78}\). The earliest ever used oxazolidinone was cycloserine (4-amino-1,2-oxazolidin-3-one), a second line drug against tuberculosis since 1956\(^ {79}\). Developed during the nineties when several bacterial strains were becoming resistant against such antibiotics as vancomycin. Linezolid (Zyvox) is first approved drug of the class.
2.2.13 Penicillins and Penicillins combinations: Penicillin (PCN or pen) is a group of antibiotics which include penicillin G (intravenous use), penicillin V (used orally), procaine penicillin, and benzathine penicillin (used as intramuscular). Origins of these are Penicillium fungi. Penicillin antibiotics were among the first medications to be effective against many bacterial infections caused by staphylococci and streptococci. Penicillins are still extensively used, though several kinds of bacteria have developed resistance due to wide use. All penicillins are β-lactam antibiotics. Penicillin was discovered in 1928 by Scottish scientist Alexander Fleming. People began using it to treat infections in 1942. There are quite a lot of improved penicillin families which are active against additional bacteria; like the antistaphylococcal penicillins, aminopenicillins and the antipseudomonal penicillins.

2.2.13.1 Mechanism of Action: Bacteria constantly remodel their peptidoglycan cell walls, simultaneously constructing and breaking down parts of cell wall as they develop and divide. β-Lactam antibiotics inhibit the formation of peptidoglycan cross-links in the bacterial cell wall; this is achieved by binding of the four-membered β-lactam ring of penicillin to the enzyme DD-transpeptidase. As a consequence, DD-transpeptidase can not catalyze development of these cross-links, and a disparity between cell wall synthesis and degradation arises, initiating the cell to swiftly die. The enzymes that hydrolyze the cross-links of peptidoglycan last to function, even while those that do not form such cross-links. This deteriorates the
cell wall of bacterium, and osmotic pressure turns out to be more and more uncompensated ultimately causing cell death (called as cytolysis).

2.2.13.2 **Biosynthesis of Penicillin G:** Overall, there are three main and important steps to the biosynthesis of penicillin G (benzylpenicillin) (Fig.-2).

1. **The first step is condensation of 3 amino acids**—L-$\alpha$-aminoadipic acid, L-cysteine, L-valine into a tripeptide\(^\text{81-83}\). In advance condensing into tripeptide, the amino acid L-valine must undergo epimerization to become D-valine\(^\text{84, 85}\). The condensed tripeptide is called $\delta$-(L-$\alpha$-aminoadipyl)-L-cysteine-D-valine (ACV). The enzyme $\delta$-(L-$\alpha$-aminoadipyl)-L-cysteine-D-valine synthetase (ACVS-a nonribosomal peptide synthetase or NRPS) is responsible for condensation reaction and epimerization both.

2. **The oxidative conversion of linear ACV into the bicyclic intermediate isopenicillin N** is second step in the biosynthesis of penicillin G by isopenicillin N synthase (IPNS) enzyme, which is encoded by the gene $pcbC$. Isopenicillin N is a very feeble intermediate, because it does not have robust antibiotic activity\(^\text{84}\).

3. **The final step is a transamidation by isopenicillin N N-acyltransferase,** in which the $\alpha$-aminoadipyl side-chain of isopenicillin N is removed and exchanged for a phenylacetyl side-chain. This reaction is encoded by the gene $\text{penDE}$, which is exceptional in progression of obtaining penicillins\(^\text{81}\).
2.2.14 Polypeptides: Polypeptide antibiotics are a chemically diverse class of anti-infective and antitumor antibiotics containing non-protein polypeptide chains. Examples of this class contain actinomycin, bacitracin, colistin, and polymyxin B. Actinomycin-D has also been beneficial in chemotherapy of cancer. Most other polypeptide antibiotics are as well toxic for systemic administration, but can carefully be administered topically to the skin as an antiseptic for superficial cuts and scratches.

2.2.15 Quinolones/ Fluoroquinolones: The quinolones and fluoroquinolones are a family of synthetic broad-spectrum antibiotic drugs. Quinolones, and
derivatives, have also been isolated from natural sources (such as plants, animals and bacteria) and can be used as natural antimicrobials or signaling molecules\textsuperscript{88}. Quinolones employ their antibacterial effect by inhibiting bacterial DNA from winding down and duplicating\textsuperscript{89}. The widely held of quinolones in clinical usage are fluoroquinolones, which have a fluorine atom attached to central ring system, characteristically at C-6 or C-7 position.

Fluoroquinolones are every so often used for genitourinary infections, and are extensively used in treatment of hospital-acquired infections related with urinary catheters. In community-acquired infections, they are suggested only when menace issues for existence of multidrug confrontation or after other antibiotic treatments have become unsuccessful. However, for serious acute cases of pyelonephritis or bacterial prostatitis where the patient may need to be hospitalized, fluoroquinolones are endorsed as first-line treatment\textsuperscript{90}. Due to sickle-cell disease patient's being at increased risk for developing osteomyelitis from the \textit{Salmonella} genus, fluoroquinolones are "drugs of choice" due to their capacity to get into bone tissue without chelating it, as tetracyclines.

\subsection{Mechanism of Action:}

1\textsuperscript{st} and 2\textsuperscript{nd} generation fluoroquinolones selectively obstruct the topoisomerase II ligase, leaving two nuclease domains undamaged. This alteration, coupled with perpetual action of the topoisomerase II in the cell of bacteria, leads to DNA disintegration via the nuclease activity of the integral enzyme territories. 3\textsuperscript{rd} and 4\textsuperscript{th} generation fluoroquinolones are further
selective for the topoisomerase IV ligase territory, and thus have enhanced gram +ve exposure.

![Fig. 3: Mode of action of Quinolones /Fluoroquinolones](image)

Fluoroquinolones can easily enter into cells via porins and, thus, are frequently used for cure intracellular pathogens as *Legionella pneumophila* and *Mycoplasma pneumoniae*. For several gram-ve bacteria, DNA gyrase is objective, while topoisomerase IV is objective for many gram +ve bacteria. Some compounds in this class have been shown to inhibit the synthesis of mitochondrial DNA\(^91-94\).

The mechanistic approach of fluoroquinolones toxicity have been accredited to their communications with diverse receptor complexes, such as blockade of the GABA\(\alpha\)\(^95\) receptor complexes within the central nervous system, leading to excitotoxicity sort of effects\(^97\) and oxidative stress\(^98\).

The quinolones divided into generations based on their spectrum of antibacterial activity\(^99, 100\). The 1\(^{\text{st}}\) generation is not often used today. Nalidixic acid was added in OEHHA Prop 65 list as a carcinogen on 15 May 1998. An integer of the 2\(^{\text{nd}}\), 3\(^{\text{rd}}\), and 4\(^{\text{th}}\)-generation drugs have been disinterested from clinical trials due to severe toxicity problem or discontinued. The drugs most often recommended
today contain Avelox (Moxifloxacin), Ciprofloxacin, Levaquin (levofloxacin), and, to some extent, their generic equivalents.

\[
\begin{align*}
\text{Ciprofloxacin} & \quad \text{Leavofloxacin}
\end{align*}
\]

2.2.16 Sulphonamides: Sulphonamide (also called sulfonamide or sulfa drugs) is the core of several sets of drugs. The innovative antibacterial sulphonamides are synthetic antimicrobial ingredients that have sulphonamide group. Few sulphonamides are also not having antibacterial activity, e.g., the anticonvulsant sultiame. Sulfonylureas and thiazide diuretics are innovative drug groups having antibacterial sulphonamides. Antibacterial sulphonamides are competitive inhibitors of dihydropteroate synthase (DHPS) enzyme, involved in folate synthesis. Hence sulphonamides are bacteriostatic and obstruct growth and multiplication of bacteria, but don’t kill them. Humans, in disparity to bacteria, acquire folate (vitamin B\textsubscript{9}) through diet\textsuperscript{101}. Sulphonamides are used to cure allergies and cough, simultaneously as antifungal and antimalarial agents. The moiety is also existent in other medicines that are not antimicrobials, including thiazide diuretics (including hydrochlorothiazide, metolazone, and indapamide, among others), loop diuretics (including furosemide, bumetanide, and torsemide), acetazolamide, sulfonylureas (including glipizide, glyburide, among others), and more or less COX-2 inhibitors (e.g., celecoxib).
Prontosil, a Bayer’s new drug, was the first drug perpetually discovered that could effectively cure a sort of bacterial infections inside body. It had a robust protective action in contradiction of infections caused by streptococci, together with blood infections, childbed fever, and erysipelas, and a slighter effect on infections due to other cocci.

Sulfonamides are synthesized by reaction of a sulfonyl chloride with ammonia or an amine. Certain sulfonamides (sulfadiazine or sulfamethoxazole) are sometimes mixed with the drug trimethoprim, which performs against dihydrofolate reductase. As of 2013, the largest exporter of Sulfonamides is Republic of Ireland worldwide, approximately 32% of total exports\textsuperscript{102}.

Sulfonamides have enough potential to cause a diversity of problematic reactions, counting porphyria, urinary tract disorders, haemopoietic disorders, and hypersensitivity etc. When used in hefty doses, they may source a resilient allergic reaction. Two of the most serious are Stevens–Johnson syndrome and toxic epidermal necrolysis (also known as Lyell syndrome)\textsuperscript{103}.

\textbf{2.2.17 Tetracyclines:} Tetracyclines are a group of broad-spectrum antibiotics whose practicality has been bargain with commencement of antibiotic resistance. To be specific, they are defined as "a subclass of polyketides having an octahydrotetracene-2-carboxamide skeleton"\textsuperscript{104}. They are derivatives of polycyclic naphthacene carboxamide.
Tetracyclines are used in the treatment of urinary tract, respiratory tract, and intestines infections and especially in patients allergic to β-lactams and macrolides.

First discovered member of the group is Chlortetracycline (Aureomycin) in the late 1940s by Benjamin Minge Duggar, a scientist of American Cyanamid - Lederle Laboratories, under the supervision of Yellapragada Subbarow; consequent substance of golden-colored fungus-like bacterium *Streptomyces aureofaciens*\textsuperscript{105}. Oxytetracycline (Terramycin) was discovered afterwards by AC Finlay et al; from bacterium named *Streptomyces rimosus*. Conovero successfully synthesized tetracycline in laboratory.

**2.2.17.1 Mechanism of Action:** Tetracycline antibiotics stop protein synthesis by inhibiting the binding of aminoacyl-tRNA to the mRNA-ribosome complex. They do so by binding to the 30S ribosomal subunit in mRNA translation complex\textsuperscript{106}.

Tetracyclines (especially chemically modified tetracyclines or CMTs - like in cyclinide) also have been found to inhibit matrix metalloproteinases for the treatment of rosacea, acne, diabetes and various types of neoplasms\textsuperscript{107-109}. A number of trials have done with modified and unmodified tetracyclines for cancers treatment; and very encouraging results were achieved with CMT-3 for patients with Kaposi Sarcoma\textsuperscript{110}.

**2.2.18 Others:** In this category multiple antibiotics are covered which are used for multiple cures. As Chloramphenicol used for the treatment of cholera, typhus, and
gram-negative and gram-positive bacteria. Fosfomycins used in treatment of acute cystitis in women. Metronidazole and Tinidazole were used in treatment of infection of anaerobic bacteria and protozoans. Side effect of these drugs may be as follows:

1. Rarely: aplastic anaemia.
2. Fosfomycin is not recommended for children and 75 up of age.
3. Tarnished urine, headache, metallic taste, nausea, alcohol is contraindicated.
4. Distressed stomach, bitter taste, and itchiness.

2.2.18.1 Mechanism of Action: Mechanism of action of such drugs is as follows:

1. Prevents bacterial protein synthesis by binding to 50S subunit of ribosome.
2. Inactivates enolpyruvyl transferase, thereby blocking cell wall synthesis.
3. Produces toxic free radicals that interrupt DNA and proteins.

2.3 Antimalarial Drugs:

All kind of malaria is transmitted by female Anopheles mosquitoes. Humans are mostly infected by 4 species of Plasmodium: P. falciparum, P. vivax, P. ovale and P. malariae, recently human infections with monkey malaria parasite, P. knowlesi have also been reported in Southeast Asia\(^{111}\). The majority of all human malaria cases are caused by P. falciparum and P. vivax and almost all severe malaria cases are reported due to P. falciparum. Sporozoite of parasite is injected into human beings when bitten by infected female Anopholes. Sporozoites rapidly enter the liver cells and multiply to form thousands of merozoites; which enter to bloodstream and attack on RBC’s and multiply to form new merozoites. Infected RBC’s rupture,
released merozoites that contaminate new RBC’s. This stage of the plasmodial life cycle exhibits symptoms of malaria.

Rate of malaria transmission is determined by parasite reservoir in a community and abundance and behaviour of mosquito carriers\textsuperscript{112}. The probability of a mosquito being infected depends on frequency, duration and density of sustainable gametocyte carriage in human host\textsuperscript{113}. There are various factors that can lead to a rise in duration and density of \textit{P. falciparum} gametocyte carriage. Gametocyte numbers upsurge with density and duration of vegetative parasitaemia (accentuating the significance of early treatment), anaemia and drug resistance\textsuperscript{113,114}.

Quinine (extracted from cinchona tree bark), member of cinchona alkaloid family, is one of the oldest antimalarial agents. Cinchona species is native to Andean region of South America. Dutch and British colonialists quickly established plantations after realising its therapeutic potential.

CQ (Chloroquine) was first produced in 1934 and turn out to be the most extensively used antimalarial drug by 1940s\textsuperscript{115}. The success of this class is due to excellent clinical efficacy, partial host harmfulness, easy to use and low cost synthesis – as little as USD 0.10\textsuperscript{116}. However, the worth of quinoline based antimalarials has been extremely battered in recent years, mainly as a result of development of parasite resistance\textsuperscript{117}. Although abundant of current research work is directed towards credentials of novel chemotherapeutic agents, we still do not completely recognise mode of action and mechanism of resistance to the quinoline derivatives that would prominently assist to design of novel, potent and economical alternative of quinoline antimalarials.
Piperaquine was first synthesized in 1960s and used extensively in China for treatment of prophylaxis. With growth of piperaquine-resistant strains of *P. falciparum* and arrival of artemisinin derivatives, its use deteriorated during 1980s\(^{118}\).

Amodiaquine 3 (AQ), a phenyl substituted analogue of CQ, was found to be active against non-human malaria in 1946. Use of AQ has been strictly restricted because of hepatotoxicity and agranulocytosis. Due to this, WHO withdrew recommendation for AQ as a monotherapy in 1990. The AQ side chain comprises of 4-aminophenol group; responsible for toxicity.

Tebuquine, a biaryl derivative of AQ discovered by Parke-Davis, is significantly potent antimalarial than AQ and CQ both. Compounds in tebuquine
series have also been revealed to have undesirable toxicity profiles that are impaired by long half-lives\textsuperscript{119}.

\begin{center}
\includegraphics[width=0.5\textwidth]{terbuquine.png}
\end{center}

Terbuquine

Pyronaridine is one more member of Mannich-base schizontocides class; however, the common quinoline heterocycle is substituted by an azaacridine.

\begin{center}
\includegraphics[width=0.7\textwidth]{pyronaridine.png}
\end{center}

Pyronaridine

While several analogues displayed potent antimalarial activity against both CQ sensitive and resistant strains, isoquine, the straight isomer of AQ, exhibited potent in vitro antimalarial activity and admirable oral in vivo ED\textsubscript{50} and ED\textsubscript{90} activity of 1.6 and 3.7 mg/kg, respectively, against the \textit{P. yoelii} (compared with 7.9 and 7.4 mg/kg for AQ)\textsuperscript{120}.

\subsection*{2.3.1. Antimalarial Resistance:}

Resistant parasites are major threat to achieve malaria control and ensuing eradication. Antimalarial resistance in \textit{P. falciparum} parasites effects in a massive
public health and economic problem. The rise in malaria related hospital admissions and malaria mortality across west, east and southern Africa for the period of the 1990’s is mostly accounted for continued use of low-priced monotherapies, chloroquine and sulfadoxine–pyrimethamine, in spite of high levels of resistance\textsuperscript{121-124}. Inferior levels of resistance are accompanying with return of illness, anaemia and increased gametocyte carriage and a greater risk of cure failure in consequent infections\textsuperscript{125, 126}. Parasite resistance has been renowned for all classes of antimalarials\textsuperscript{127, 128}. If the efficiency of artemisinin derivatives is lost, then effective control and elimination will not be possible with currently available tools\textsuperscript{129}. In spite of these concerns, artemisinin-resistance phenotype has been below par considered, and involvement of host factors remnants to be distinct. The key features of the artemisinin-resistant phenotype are elongated parasite clearance times, notwithstanding superficially suitable drug exposure, and even dose escalation\textsuperscript{130, 131}. A genetic basis for this clinical phenotype has been projected in recent times on its high heritability\textsuperscript{132}.

The recent regressions in the clinical efficacy of all antimalarial drugs have impelled suggestions to look over the definitions of antimalarial drug resistance to include a category for extensively drug-resistant (XDR) malaria, as this approach has evidenced beneficial for tuberculosis in patients\textsuperscript{133}.

Drugs with extended mortal elimination half-lives lead to a longer post-treatment prophylactic consequence, which seems to be significant for their action in intermittent preventive therapy (IPT) in high-risk groups. However, these extensive acting antimalarials have drawback of residual concentrations deterring sensitive parasites more than resistant parasites, thus stimulating the resistance. (Fig. 4).
Fig.-4: Resistance choice by drugs with extended elimination half-lives. The dotted line curves show drug concentrations for Drug A and solid line for Drug B over time. Window of choice is time when antimalarial concentrations are enough to clear sensitive but not resistant parasites. Hypothetical minimum parasiticidal concentrations (MPCs) required to clear sensitive, partially resistant (Res 1) and highly resistant (Res 2) parasites shown by 3 dotted lines.

Antimalarial resistance feasts because gametocyte carriage and infectivity to mosquitoes is constantly higher in patients infected with drug-resistant parasites. A rise in number of gametocytes has been acknowledged as first sign that an antimalarial is start to fail and stresses the need of implementing drugs that will kill sexual phases\textsuperscript{135}. Combining antimalarials with contrary modes of action is anticipated to reduce the chances of a resistant parasite persisting treatment\textsuperscript{136}.

An added challenge to restraining rate of spread of ACT resistance is that escalating ACT access is essential for reducing malaria disease and impermanence.
To be accessed on time, ACTs necessity to be available near home. With recent efforts of cost reduction, ACTs becomes feasible even in underprivileged communities. However, such ready access creates the opportunity for widespread and indiscriminate use of antimalarials, which employs a strong selective pressure to resistant parasites towards great intensities of resistance\textsuperscript{130}. This could be talked by regulating ACT use to peoples with confirmed malaria diagnosis\textsuperscript{137}.

Other challenges to the effective targeting of ACTs are that only 38 countries (16 in Africa) are organising quick diagnostic tests at community levels and that ACTs continue to be used by those with negative malaria tests\textsuperscript{138, 139}.

From start antimalarial drug resistance is most probable to occur in manic parasitaemic patients who are non-immune, predominantly if their antimalarial drug acquaintance is poor\textsuperscript{140}. In hyper-parasitaemic patients, parasite populations are larger and recrudescence rates following treatment are high\textsuperscript{141}. Drug exposure can be poor due to sub-standard quality drug, poor obedience, and vomiting, unusual pharmacokinetic behaviour or under dosing\textsuperscript{134}. Recommending nethermost effective dose, while justified for cost and safety / tolerability, might not be sensible choice. For example, mathematical modelling recommends that, if 25-mg/kg Mefloquine dose had been arrayed initially, as a substitute of 15 mg/kg, then mefloquine resistance could be hindered\textsuperscript{142}. This approach emboldens resistance selection, mainly in patients with high parasite problems and low drug intensities\textsuperscript{140}. Often young children or pregnant women have deficient immunity and greater parasite concentrations, result in sub-optimal drug concentrations for many antimalarial drugs\textsuperscript{134, 140}. In spite of wide use for 4 decades, it has been acknowledged that currently suggested doses of both sulfadoxine–pyrimethamine and chloroquine attain
significantly low plasma drug concentrations in young children than older patients\textsuperscript{143, 144}.

2.4 Literature reviewed for Pyrazole as Antimicrobial Agents:

1. Many natural and synthetic product containing pyrazole\textsuperscript{145, 146}, were reported to possess varied pharmacological activities. Many of these biological activities were attributed to the presence of N-bridge heterocyclic nuclei of pyrazole, which are described to have antiviral and antimicrobial activity\textsuperscript{147}. On the basis of this study F. E. Goda et al synthesized following structured derivatives and studied their antimicrobial activities\textsuperscript{148}.

2. The target compounds synthesized by A.A. Bekhit et al. were evaluated for their antimicrobial activity against \textit{E.coli} representing Gram-negative bacteria, \textit{S. aureus} representing Gram-positive bacteria and \textit{C. albicans} representing fungi. Microdilution susceptibility test in Muller-Hinton broth (Oxoid) and Sabouraud liquid medium (Oxoid) were used for determination of antibacterial and antifungal activities\textsuperscript{149}. The minimal inhibitory concentrations (MIC) showed that all the tested compounds have weak or no antifungal activity against \textit{C. albicans} except few compounds showed half the activity of the antifungal drug used (clotrimazole). Regarding the antibacterial activity few compounds displayed comparable antibacterial activity against \textit{E. coli} to that displayed by the reference antibacterial drug used (ampicillin)\textsuperscript{150}.
3. Substituted oxazole, pyrazole and their analogs was used as precursors for synthesis of various biologically active molecules, oxazole derivatives as brain-derived neurotrophic factor inducers\textsuperscript{151}, analgesic\textsuperscript{152}, antifungal activity\textsuperscript{153}, anti-inflammatory\textsuperscript{154}, antidepressant\textsuperscript{155}, antimicrobial, antidiabetic and antiobesity\textsuperscript{156-157}. Oxazolidinones have been acknowledged as new class of orally active synthetic antibiotics with a unique mechanism of bacterial protein synthesis inhibition. Literature survey reveals that less attention is given to the synthesis of oxazole nucleus having pyrazole link. N. D. Argade, K. Kalrale and H. Gill did the synthesis of pyrazoles containing 2,4-disubstituted oxazoles as a new class of antimicrobial agents\textsuperscript{158}.

4. Bufuralol is a non-selective b-adrenoceptor antagonist developed by Hoffman La Roche\textsuperscript{159}. On the other hand, compounds including pyrazole nucleus are known to possess analgesic, anti-inflammatory, antipyretic, antiarrhythmic, tranquilizing, muscle relaxant, psychoanaleptic, anticonvulsant, hypotensive, monoamine oxidase inhibitor, antidiabetic, and antimicrobial activities\textsuperscript{160-164}. On the basis of earlier
studies Bakr F. Abdel-Wahab, Hatem A. Abdel-Aziz, and Essam M. Ahmed synthesized following derivatives which are noticeable antibacterial activities. The inhibition zones of compound A against Bacillus subtilis are larger than that of the control Amoxicillin. While compound B showed antibacterial activities more than the control against E. coli.165

Literature survey revealed that thiazole, pyrazole and thiophene ring structures have employed a unique position in the design and synthesis of novel biological active agents with remarkable analgesic and anti-inflammatory activities166-169, in addition to their well-documented potential antimicrobial activities170-173. Fourteen of the newly synthesized target compounds were evaluated for their in vitro antibacterial activity against Staphylococcus aureus (ATCC 25923) and Streptococcus pyogenes (ATCC 19615) as examples of Gram-positive bacteria and Pseudomonas phaseolicola (GSPB 2828) and Pseudomonas fluorescens (S 97) as examples of Gram-negative bacteria. They were also evaluated for their in vitro antifungal potential against Fusarium oxysporum and Aspergillus fumigatus fungal strains174. Out of these 14 compounds pyrazole derivatives are as follows:
6. All the synthesized pyrazole compounds were screened for antibacterial activity against three Gram +ve strains and three Gram -ve strains by MTT technique. The antibacterial activity of reference compounds kanamycin B and penicillin G were also included. Studies were performed by modification of the A ring and B ring of the parent compounds to regulate how substituents of subunits affected antibacterial activities. Few compounds displayed potent activity with MIC values of 0.39 \( \mu \text{g/mL} \) and 0.78 \( \mu \text{g/mL} \) against \( E. \text{coli} \), which were superior to the positive control kanamycin B. Few compound exhibited significant activity with MIC values of 1.562 \( \mu \text{g/mL} \) against \( E. \text{coli} \), which was comparable to the positive control kanamycin B. Based on the data obtained, we found that compounds bearing a methoxy group at 4-position at A ring showed potent antibacterial activities against \( E. \text{coli} \). Among them, a comparison of the substitution on B-ring demonstrated that 4-position-substituted derivatives with halogen atoms have more potent activity against \( E. \text{coli} \). Most significantly, the stronger electron-withdrawing substituents the compound contained on B-ring at 4-position is more potent, which was demonstrated by potency order \( \text{F} > \text{Cl} > \text{Br} \). Meanwhile, the derivatives which have electron-donating substituents (such as CH3, OCH3) on B-ring exhibited less potent activity (MICs values - 3.125 \( \mu \text{g/mL} \) to 25 \( \mu \text{g/mL} \))\textsuperscript{175}. 

![Chemical Structure](image)

7. Few of all these synthesized compounds were exhibiting pronounced activity against \( P. \text{marneffei} \) and \( T. \text{mentagrophytes} \). Some compounds were more active against \( A. \text{fumigates} \) whereas some other were moderately active. Some derivatives
were not active against *A. fumigates*. Some compounds were most active against *A. flavus*, whereas some shown moderate activity, and all the other compounds found to be less active\(^\text{176}\).

![Chemical structure](image)

8. A novel series of substituted 3,5-diphenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide have been synthesized by treating substituted \((2E)\)-1,3-diphenylprop-2-en-1-one derivatives with thiosemicarbazide. The synthesized compounds were investigated for antimicrobial activity. Antibacterial activity of the test compounds was evaluated against two stains, Gram-positive bacteria, *Staphylococcus aureus* and Gram–negative bacteria, *E.coli*. Ofloxacin was used as standard drug. Antifungal activity was evaluated for *Aspergillus niger* and *Penicillium chrysogenum*. Ketoconazole was used as standard drug. Maximum inhibitory activity is observed in Gram-positive bacteria *Staphylococcus aureus*. Few compounds had shown best activity due to presence of p-methoxy, dimethoxy, trimethoxy, o-nitro and p-chloro. In the case of Gram-negative *E.coli* the zone of inhibitory activity was not much significant because of a multilayered phospholipidic membrane carrying the structural lipo-polysaccharide components 36 and among all the compounds only one was found to be most active when compared with std. drug (Ofloxacin) at both the concentrations because of possession of amino group on ring I\(^\text{177-178}\).
9. The in vitro antibacterial activity of newly synthesized compounds was determined by well plate method. In this work, *E. coli, S. aureus, B. subtilis, S. typhimorium, C. profingens* and *P. aeruginosa* were used to investigate the activity. Test compounds were liquefied in dimethyl sulfoxide (DMSO) (concentrations - 1 and 0.5 mg/mL). The antibacterial screening revealed that some compounds showed good inhibition against various microbial strains. The result of tested compounds indicated, one (as shown below) showed excellent activity against *P. aeruginosa* at concentrations of 1 and 0.5 mg/mL compared to streptomycin. Same showed similar activity as that of standard, against *C. profingens*, at 1 and 0.5 mg/mL concentrations.

Newly synthesized compounds were also screened for their antifungal activity against *A. flavus, A. niger, C. albicans, M. gypseum*, and *T. rubrum*. Among the tested compounds, same has emerged as active against *T. rubrum* compared with standard, fluconazole, whereas other compounds showed less activity against all the tested microorganisms compared to standard drug.

10. All the newly synthesized carboxylic acids and carbothioamides were assessed for in vitro antibacterial activity against *S. aureus* (MTCC 96) and *B.*
subtilis (MTCC 121) signifying Gram +ve bacteria, and *E. coli* (MTCC 1652) and *P. aeruginosa* (MTCC 741) representing Gram -ve bacteria by agar well diffusion method using ciprofloxacin as the reference drug. By calculating zone of inhibition against test bacterium, compounds A and B were found to be the most effective against *S. aureus* with high zone of inhibition (21.6 mm) in compare with established drug (75.3% and 74.6% inhibition), and few compounds showed modest antifungal activity (55% inhibition of mycelial growth) against *A. niger* and *A. flavus* respectively.

11. The six tested bacterial strains, *S. aureus*, *S. faecalis*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *N. gonorrhoeae* were inhibited mostly by compounds (A) at MIC 20 mg/ml. On the other hand, only *N. gonorrhoeae*, *S. faecalis*, and *E. coli* were inhibited by chalcone (B). Thus, bacteria *N. gonorrhoeae*, *S. faecalis*, and *E. coli* were observed to be more sensitive than *S. aureus*, *B. subtilis*, and *P. aeruginosa*. Only *E. coli* were found to be sensitive towards compound (C)
12. Compounds of series 4, 5, and 6 show excellent activity against the strains of bacteria. The substituents (-OH, -Cl) for the compounds of series 4, 5 and 6 are important for biological potency. 

13. All compounds were screened in vitro for their antimicrobial activity against, by agar diffusion method. The antibacterial activity of the synthesized compounds was tested against *B. subtilis*, *S. aureus* (Gram-positive bacteria), *E. coli*, *Pseudomonas sp.* (Gram-negative bacteria) using nutrient agar medium. Antifungal activity of compounds was confirmed against *C. albicans*, *A. niger* and *Pencillium sp.* by Sabouraud dextrose agar medium. The result revealed that compounds showed varying degrees of inhibition against the tested microorganisms. In general, the best antibacterial activity was displayed below, which showed strong antibacterial activity and good antifungal activity.

14. All of our compounds, i.e. pyrazoles, pyrazolines were assayed for antimicrobial activity contrary to 4 organisms *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans* using rifampicin (5 µg/disc) and ampicillin (10 µg/disc) as standard drugs. The antimicrobial activity results of our synthesized derivatives against *S.*
*aureus* and *C. albicans* showed the trend of activity as follows: X > H > OMe > NO2 where X = Cl, Br. Minimum batericidal concentrations (MBC) were determined for all chloro derivatives exhibit good activities.184

![Chemical structure](attachment:structure.png)

15. All the newly synthesized compounds were screened for their antimicrobial activity. Antimicrobial study was assessed by Minimum Inhibitory Concentration (MIC) by the serial dilution method. For this, *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa* microorganisms were used to investigate the antibacterial activity. Antifungal activity was carried out against *C. albicans*. Ceftriaxone and Fluconazole were used as standard drugs for antibacterial and antifungal activities respectively. Among the screened samples, (A) emerged as potent antimicrobial agents. Compound (A) contains 2,5-dichlorothiophene substituent on a pyrazole ring which accounts for the enhanced activity. Some derivatives have 2,4-dichlorophenyl moiety and 2,5-dichlorothiophene substituent correspondingly, which is responsible for the enhanced activity of the compounds. Antifungal activity data revealed that few compounds had presented parallel activity against fungal stain *C. albicans* compared to the standard drug Fluconazole.185

![Chemical structure](attachment:structure.png)

16. The newly synthesized compounds were evaluated for their antibacterial and antifungal activities and compared with reference drug Ofloxacin and Fluconazole.
Among the synthesized compounds few were found equipotent active against different stains. Compounds bearing carboxyl and chloro substituents at \textit{para} position of phenyl ring showed excellent antibacterial results. Compounds with \textit{para}-fluoro substituent of phenyl ring showed excellent antifungal results\textsuperscript{186}.

17. The antibacterial screening for isolated compounds was determined by the disc diffusion method using Mueller–Hinton agar (Hi-Media) medium. The synthesized compounds were assessed against gram -ve bacteria of \textit{E. coli}, \textit{P. aeruginosa}, \textit{K. pneumonia} (recultured) and gram +ve bacteria of \textit{S. aureus}. Compound (R = OH) is exceptionally active (MIC: 0.25 \textmu g/mL) against gram -ve bacteria of \textit{E. coli} compared with Ciprofloxacin MIC: 0.5 \textmu g/mL. Compound (R = \textit{NO}_2) (MIC: 0.25 \textmu g/mL) is very much active as standard (MIC: 4 \textmu g/mL) against gram -ve bacteria of \textit{S. epidermidis}. Compound (R = Cl) MIC: 1 \textmu g/mL is significantly active against \textit{A. niger} as Clotrimazole MIC: 2 \textmu g/mL. Compound (R = Cl) (MIC: 0.5 \textmu g/mL) has equipotent activity to Clotrimazole (MIC: 0.5 \textmu g/mL) against \textit{M. audouinii}\textsuperscript{187}.

\[ R = \text{OH}, \text{NO}_2, \text{Cl} \]
2.5 Literature reviewed for Indole as Antimicrobial Agents:

1. The key pharmacophore (IC50=2.7 mM) was determined to be 2-benzoyl-1,2,3,4-tetrahydro-9H-pyrido[3,4-b]indole. Increased inhibition of both *S. aureus* and *E. coli* was observed for 4-substituted benzyl analogues, with the 4-hydroxybenzyl compound exhibiting the greatest potency followed by 4-fluorobenzyl compounds.  

   ![Chemical Structure](image1)

2. Preliminary antimicrobial susceptibility tests for all the synthesized indole derivatives were performed by using cup plate method at a concentration of 250 μg/mL against some selected pathogenic strains. *S. aureus, E. coli, P. vulgaris, K. pneumoniae* were used for bactericidal activity and *A. fumigatus, C. albicans, C. crusei* for fungicidal activity. On the basis of structure activity relationship, it is concluded that incorporation of S atom brings an increase in bactericidal inhibition. Azomethine linkage enhances antibacterial and antifungal activity. *p*-Methoxy substituted azetidinone and *o*-hydroxy substituted thiazolidinone moieties claim most potent antibacterial and antifungal activity.

   ![Chemical Structure](image2)

3. The antibacterial activity of compounds against MRSA standard and MRSA isolate showed promising results when it was compared to the control drug
ampicillin. Few compounds with an MIC value of 6.25 µg/ml had shown more strong antimicrobial activity than ampicillin (MIC is 12.5 µg/ml). Also few showed moderate activity against MSRA standard and MSRA isolate. Against *B. subtilis* with the exception of one compound, all the compounds indicated more potent or similar activity than that of ampicillin. Few compounds showed moderate activity against *B. subtilis* compared to ampicillin. Among the tested compounds one showed good antifungal activity against *C. albicans* with an MIC value of 3.125 µg/ml which was close to that of fluconazole (MIC 0.78 µg/ml)\(^{190}\).

\[ \text{R} \quad \text{HN-Ar} \quad \text{HN} \quad \text{Ar} \]

4. The in-vitro antimicrobial activity testing was performed by using the cup-plate method. All the fused quinazolines prepared herein were screened for their potential antibacterial activity against *S. aureus, B. subtilis, S. pyogenes* (Gram-positive) and *S. typhimurium, E. coli, K. pneumonia* (Gram-negative) bacterial strains. Ampicillin was used as a reference standard. All the newly synthesized fused quinazolines compounds were also examined for their antifungal activity against the strains *A. niger, C. albicans, T. viridae*. Ketoconazole was used as standard drug for comparison of the antifungal results. The antimicrobial screening revealed that all the tested compounds showed moderate to good inhibition towards all bacterial and fungal strains. The maximum inhibition was observed in one compound against all the tested organisms except *S. typhimurium*. In addition, the most potent activity against all bacterial and fungal strains was observed in two fused quinazolines comparing them with the respective standard drugs ampicillin and ketoconazole\(^{191}\).
5. Antimicrobial Activity was observed at 50 mg/mL of concentration. The antimicrobial activity was observed in compound 1 (against *B. subtilis* and *P. aeruginosa*), 2 (against *S. aureus, E.coli* and *B. subtilis*) and 5 (against *S. aureus*). No compound was found active against *A. niger*. Only three were found effective against bacterial strains at a much higher concentration as compared to the standard drug, Norfloxacin and none of the synthesized compound was found effective against fungal strain.

![Diagram](attachment:image.png)

6. All compounds and standard drug chloroamphenicol antibacterial activity was carried out against *E. coli* ATCC 25922, *B. subtilis* ATCC 1633, and *S. aureus* ATCC 25923. From the results it is clear that compounds with structure-2 (shown below) have excellent antibacterial activity (MIC: 3.125 µg/mL) better than standard drug. Compounds with structure-2 and compounds with structure-3 exhibited higher activity against *E. coli* and *S. aureus* than the standard drug with MIC: 6.25 µg/mL. Further, several compounds exhibited antibacterial properties equipotent to reference drugs. All compounds tested for antifungal activity with reference drug fluconazole against *C. albicans* ATCC 2091, *A. niger* ATCC 9029, and *C. krusei* ATCC 6518, revealed that all tested compounds showed moderate to good antifungal activity. Compounds with general structure-2 (MIC values: 3.125, 6.25, and 1.592 µg/mL) were found to be more potent antifungal agents against *C.*
albicans, A. niger, and C. krusei than the reference drug. The other compounds of this series were less active compared to the standard drug.

7. All newly synthesized azomethines were assayed for antibacterial activity against S. aureus, B. subtilis, S. pyogenes (Gram +ve); S. typhimurium, E. coli and K. pneumonia (Gram -ve) bacterial strains in addition to their antifungal activities against A. niger, C. albicans and T. viridae. In vitro antimicrobial activity was executed by a cup-plate method (Reddy et al., 2009; Rohini et al., 2009b). Ampicillin and ketoconazole were used as standards in this assay. Primary screening of all azomethines was executed at 1000 μg/mL. The preliminary screening data revealed that all the tested compounds Az1-13 showed moderate to good inhibition towards all tested strains. Mainly, high zone of inhibition values were observed for the Az5, Az6 azomethines for all tested strains. Furthermore, the majority of bis-azomethines showed most strong activity against all bacterial and fungal strains as compared to standards. From this study, it is clear that Az5, Az6 compounds (MIC: 5-20 μg/mL) and especially the bis indole azomethines (MIC: 2.5-20 μg/mL) exhibited greater activity against all bacterial and fungal strains. In conclusion, we have synthesized thirteen novel indole azomethines by using 2-(o-aminophenyl)indole and diketones (R-CO-R1/R-CO-X-CO-R1). The compounds which contain Pyridyl substituents and two azo (-C=N) groups in their structures (bis-azomethines) look to be more potent as compared to standards like ampicillin and ketoconazole. However, the method of action of these compounds is unknown.
These observations may promote an additional research in this field. Further development of this group of compounds may lead to compounds with better pharmacological profiles than the standards and serve as templates for the construction of better drugs to combat bacterial and fungal infections\textsuperscript{194}.

![Chemical structure](image)

8. All compounds were screened for their in vitro antimicrobial activities to determine zone of inhibition at 100 $\mu$g/ mL against 3 Gram +ve bacteria (\textit{S. aureus} MTCC 096, \textit{B. subtilis} MTCC 441 and \textit{S. epidermis} MTCC 435), four Gram -ve bacteria (\textit{E. coli} MTCC 443, \textit{P. aeruginosa} MTCC 424, \textit{S. typhi} MTCC 733, and \textit{K. pneumoniae} MTCC 432) as well as four fungi (\textit{A. niger} MTCC 282, \textit{A. fumigatus} MTCC 343, \textit{A. flavus} MTCC 277, and \textit{C. albicans} MTCC 227) using cup plate method. Ciprofloxacin and Miconazole were used as positive control. The obtained results revealed that spiroindoles and bis- spiroindoles could effectively, to some extent, inhibit the growth of all tested strains in vitro. In antibacterial studies, all the compounds tested were less active towards \textit{S. epidermis} and \textit{P. aeruginosa} in compared to other five strains of bacteria, whereas all the compounds showed moderate to comparable activity against \textit{S. aureus} and \textit{K. pneumonia}. Out of four strains of fungi, these spiroindoles were showed significant activity against \textit{A. niger} and \textit{C. albicans}, whereas moderate to mild activity against \textit{A. fumigatus} and \textit{A. flavus}. The prepared mono spiroindoles demonstrated moderate to good antimicrobial activities towards the tested microorganisms. Noticeably, the fluoro
derivative of spiroindoles gave comparably better activity than others against all the microorganisms. These showed that halogen comprising heterocyclic compounds exerted important influence on antimicrobial activities. It was particularly pointed out that, among these tested bis-spiroindoles linked via (CH2)6 linker possessed significant antimicrobial activities with respect to standard drugs and it gave the better zone of inhibition value in compare to mono spiroindoles. Moreover, the antimicrobial potency for spiroindoles depended on the substituent and lengths of aliphatic chains, and the activities seemed to decrease with the increase of aliphatic chain length\textsuperscript{195}. 

\begin{center}
\includegraphics[width=\textwidth]{diagram.png}
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