Chapter 1

Introduction
Various pathogens cause opportunistic infections that take advantage of a suppressed immune system. Also conditions such as HIV/AIDS disease, organ transplantation, and long-term use of corticosteroids, for example, cause either immune suppression or some disruption in the immune system [1]. With an estimated 40.3 million people living with AIDS around the globe [2] and the increasing development of resistance to current therapies, there is a continuing need for new anti-infective agents against opportunistic infections [3]. In present scenario tuberculosis, malaria and HIV/AIDS are called the “big three” killer diseases and are responsible for severe morbidity and mortality, especially in developing countries where control interventions are inaccessible, unaffordable and plagued by widespread drug resistance [4, 5]. The current chemotherapy is based on age old drugs like Isoniazid, Pyrazinamide and Rifampin for tuberculosis [6] and Pyrimethamine-sulfadoxine, Chloroquine for malaria [7, 8]. But, the efficacy of these drugs has been deteriorated by the emerging resistant strains. More over the pathogenic synergy of HIV to these diseases is alarming the world to develop new efficient chemotherapy for infectious diseases [9-11]. Thus, the ever-increasing drug resistance, toxicity, side effects of currently used drugs, and the absence of their microbial activity highlight the need for new, safer, and more effective anti-infective compounds [12]. Specifically since no effective vaccine is available, the major strategy to combat the spreading of TB/malaria is not only chemotherapy [13], but also to develop new agents with potent sterilizing activity with short duration [14].

Antibiotics are among the most prescribed therapies, in both hospital and outpatient settings. An estimated 40 percent of outpatient prescriptions written for children younger than 10 years of age are for antibiotics, and anti-infective agents account for upto 50 percent of the formulary budgets of pediatric hospitals [15, 16]. Given our reliance on these agents, it is not surprising that over the last three decades a proliferation of numerous antimicrobial agents and classes of agents has occurred. Although this proliferation has been instrumental in improving our ability to combat antimicrobial resistance, the use and misuse of available agents have contributed significantly to the continuing problem of antimicrobial resistance.
1.1 Anti-infective agents and its selection

The selection of an antimicrobial regimen for the treatment of a specific infection must take into account many important factors. These factors can be divided into those pertaining to the microbiology & anatomy of the infection, those pertaining to the specific antimicrobial agent, and those relating to the host.

1.1.1 Microbiological and Anatomic Factors

Ideally, the choice of an antimicrobial agent is based on a documented infection, in which a pathogen is identified and the *in vitro* susceptibility profile is known. However, because antimicrobial therapy most often is initiated empirically, identifying the site of infection and having a thorough knowledge of the likely pathogens causing infection at that site are critical. Equally important knows the susceptibility patterns for the suspected pathogens, preferably based on contemporary local community and hospital data. In general, initial empiric therapy should include broad coverage against the likely pathogens, which must take into account the site and severity of the infection. For example, a life-threatening infection, such as bacterial meningitis, requires that the antimicrobial agent provide coverage against all suspected pathogens, including potentially highly resistant ones. However, a less serious infection in which the natural course often is favorable even without antimicrobial therapy (eg, otitis media) may warrant coverage against only the most likely pathogen. Once microbiologic and *in vitro* susceptibility data are obtained, the antimicrobial regimen can be narrowed. If a pathogen is never identified, as frequently is the case, the presumed antimicrobial susceptibility pattern and the clinical response should guide subsequent therapy.

The site of the infection often will dictate which agents can be used. Agents that penetrate the central nervous system (CNS) should be used for the treatment of meningitis or bacteremia. Some infections, such as meningitis and endocarditis, require treatment with agents that are bactericidal, whereas others, such as osteomyelitis, can be effectively treated with bacteriostatic agents; clindamycin is ineffective for the treatment of *staphylococcal endocarditis*, but it is an excellent choice for the treatment of *staphylococcal osteomyelitis*. 
The decision to initiate single versus combination therapy often depends on the specific microbiological characteristics of a particular infection. Indications for combination therapy include the treatment of polymicrobial infections, the prevention of the emergence of resistance, possible synergistic effect, and the empiric therapy for possibly resistant gram negative infections in the immune-compromised host. The combination of penicillin and an aminoglycoside has been shown to be synergistic for the treatment of enterococcal endocarditis and neonatal group streptococcal sepsis [17, 18] Similarly, this combination frequently is used to provide synergy and to prevent the emergence of antimicrobial resistance in the treatment of serious infections caused by Pseudomonas aeruginosa and Klebsiella pneumonia in the immunocompromised host [19, 20].

1.1.2 Drug Factors

The safety profile, pharmacokinetics, and pharmaceutical properties of a particular agent often are key factors in the selection of an antimicrobial agent. Whenever possible, an agent with a longstanding proven safety, record should be used, rather than a novel agent with a safety profile that has not been established over a long period.

Pharmacokinetic properties play critical roles in antibiotic selection. The need for agents that penetrate a specific site of infection, such as for meningitis, is obvious. Likewise, to avoid specific toxicity the choice may be influenced by the agent's mode of elimination.

- Bio-availability
- Tissue penetration
- Protein binding
- Mode of elimination

Knowledge of the bioavailability of an agent often influences not only the choice, but also the route of administration of an antibiotic. For example, the oral administration of trimethoprim sulfamethoxazole, metronidazole, and fluconazole results in serum levels comparable to those achieved when these agents are administered intravenously. Oral chloramphenicol administration results in higher serum levels than does intravenous administration.
The pharmaceutical properties of an antibiotic may affect its usefulness. For instance, dicloxacillin has the best *in vitro* activity against sensitive *Staphylococcus aureus*, but its liquid preparations are not well tolerated because of poor taste and low concentration.

1.1.3 Host Factors

Regardless of microbiological and drug considerations, the choice of an antimicrobial agent often is limited by patient related factors. Age, drug allergies, underlying disorders, altered renal or hepatic metabolism, prior response to therapy, and the likelihood of good compliance are but a few of these complex factors. The age of the patient is critical when considering potential pathogens and, thus, influences antimicrobial choice. Some antimicrobials are not recommended for certain age groups. For example, the sulfonamides are not recommended in premature infants and neonates because of their propensity to compete for bilirubin binding sites on albumin. Similarly, the likelihood of good compliance may dictate the choice of an antimicrobial agent by limiting the choice to agents that require less frequent dosing intervals. These are only a few examples of the patient related factors that come into play every time an antibiotic is prescribed.

Consideration of the characteristics of the host; the likely pathogen involved; the site and severity of the infection; and the pharmacokinetic, pharmaceutical, and safety profiles of an agent form the basis of appropriate antimicrobial selection.

1.2 Anti-infective drugs & its development

With the introduction of the sulphonamides in the 1930s, followed by penicillin in the 1940’s, the antimicrobial era had begun. Considering one of the most important events in medical history (Fig. 1), the discovery of antibiotics revolutionized the field of infectious diseases by giving the physicians the ability to prevent, cure and reduce transmission of certain diseases. Consequently, a significant reduction in morbidity and mortality associated with infectious disease had been achieved [21].
Fig. 1
The success of penicillin in the 1940s led researchers to intensify the search for new antibiotics that could treat other bacterial diseases. Therefore, during the 1940s to the beginning of the 1970s, the development and production of antimicrobial compounds was very successful, resulting in several new classes of antibiotics. However, during the 1970s the production of antibiotics declined, and it took almost 30 years before a new class of antibiotics the oxazolidinones [22, 23] was introduced on the market. Instead, during this period of time, the new antibiotics introduced primarily consisted of chemical modifications of already known compounds.

1.2.1 β-lactam Antibiotics
The β-lactam antimicrobial agents are defined by the presence of β-lactam ring in their basic structure. The nature of the side chains and other rings that are directly linked to the β-lactam ring determines the specific class of antibiotic, the antimicrobial spectrum, and the pharmacological characteristics.
In general, these agents inhibit numerous enzymes (penicillin-binding proteins) that are involved in the synthesis of the bacterial cell wall. These agents are the most frequently prescribed antimicrobial agents and often the first-line agents for pediatric infections, a reflection of their excellent safety profile and the fact that they are bactericidal against most pediatric pathogens.

The spectra of activity and clinical pharmacology of the β-lactam agents are quite variable and will be considered within each class of agents. However, because of adverse effect common to all the β-lactam agents.

1.2.1.1 Penicillins

They were the first in this group of antibiotics to be discovered and generally effective against gram positive bacteria, but groups of penicillins that were effective against gram negative bacteria were later discovered, and these proved to be potent broad-spectrum antibiotics, especially when combined with β-lactamase inhibitors.

1.2.1.2 Cephalosporin

This is a group of antibiotics with bactericidal effect. This is achieved by inhibition of peptidoglycan that is needed for cell wall synthesis. The first generation of cephalosporins is primarily effective against gram positive bacteria, but the later second and third generations are more effective against gram negative bacteria. Fourth generation cephalosporins are broad spectrum antibiotics with activity against both gram negative and gram positive bacteria.

1.2.1.3 Carbapenem

Carbapenem have a chemical structure that makes them highly capable of withstanding β-lactamases. They have the broadest antibacterial spectrum of the β-lactam antibiotics. They are active against both gram positive and gram negative bacteria, but not to intracellular bacteria.

1.2.1.4 Monobactam

Monobactam are synthetic monocyclic β-lactam antibiotics, derived from a bacterium. They are inactivated by some β-lactamases and by all extended spectrum beta-lactamases.
(ESBLs). They are mainly used in *P. aeruginosa* infections, but they are also active against *Enterobacter* spp, *Serratia* spp, *E. coli*, *Klebsiella* spp, *Haemophilus* spp, *Proteus* spp and *Citrobacter* spp.

1.2.2 **Aminoglycoside**

Aminoglycosides are derived from Streptomyces or Micromonosporas. In the former case they are given suffix –mycin and in the latter –micin. They bind to a sub-unit of the ribosome and block initiation of protein synthesis. They also makes mRNA misread, which also inhibits protein synthesis. In high doses they have dose dependent nephro and ototoxic effects, and therefore serum concentrations have to be monitored carefully. Aminoglycosides are used predominantly to treat infections with aerobic Gram negative bacteria such as *Enterobacter* spp, *P. aeruginosa* and *Acinetobacter* spp.

1.2.3 **Macrolide**

Macrolides have a lactone ring to which deoxy sugars are attached. Their main effect is bacteriostatic but in high concentrations they can also be bactericidal. They exert their effect by binding reversibly to the ribosome in the bacteria, inhibiting protein synthesis. They are mainly eliminated through the liver. Macrolides are mainly effective against Gram positive bacteria but not *Enterococcus* spp.

1.2.4 **Vancomycin**

Vancomycin is a tricyclic glycopolypeptide that was first introduced in the mid 1950s because of its effectiveness against penicillin-resistant staphylococci. Its use diminished with the advent of more effective and safer antistaphylococcal agents. Vancomycin has multiple mechanisms of action, which likely accounts for its relatively low frequency of development of resistance. It inhibits cell wall synthesis of gram-positive bacteria, but at a stage different from that of the β-lactam agents. It also alters the permeability of the cell membrane and selectively inhibits RNA synthesis.

1.2.5 **Quinolone**

The quinolones are derivatives of nalidixic acid, first synthesized in 1962 and used for the treatment of gram negative urinary tract infections. These agents interfere with bacterial replication by inhibiting DNA gyrase. The newer quinolone agents (eg,
ciprofloxacin and norfloxacin) contain a fluoroide atom in their structure, which results in bactericidal activity against gram-negative organisms, including *P. aeruginosa*, as well as some gram positive cocci. These agents do not have reliable activity against *staphylococci, streptococci*, or anaerobes. Ciprofloxacin is the only quinolone for which pediatric pharmacokinetic and clinical data are available. This agent is available in parenteral and oral formulations. The oral formulation is not available as a suspension and is limited by its bitter taste.

### 1.2.6 Clindamycin

Clindamycin and its parent drug, lincomycin, are lincosamide agents, dissimilar in structure from any other antibiotic. These compounds inhibit bacterial protein synthesis by binding to the 50S subunit of the ribosome, closely resembling the mechanisms of erythromycin and chloramphenicol. Clinically, clindamycin has replaced lincomycin because of superior antimicrobial spectrum and oral absorption. Clindamycin is very effective against most strains of *staphylococci, streptococci*, and pneumococci. Methicillin-resistant strains of *staphylococci* may not be susceptible, however. Enterococci and gram negative aerobic organisms are resistant. An important feature of clindamycin is its excellent activity against most anaerobes, including *Bacteroides* species.

### 1.2.7 Cotrimoxazole

The combination of trimethoprim and sulfamethoxazole has resulted in an agent with additive or synergistic effects against numerous important pediatric pathogens. Sulfamethoxazole works by competitively inhibiting the incorporation of para-aminobenzoic acid into folic acid, whereas trimethoprim subsequently inhibits the reduction of dihydrofolate to tetrafolate. This combination is active in vitro against many gram negative aerobes, including *E coli, K pneumoniae*, *Proteus* sp, *H influenzae, M catarrhalis*, and *Shigella* sp, but it has variable activity against gram positive organisms such as *S aureus* and *S pneumoniae*. The combination is not active against *P aeruginosa* or groups *A streptococci*. It has excellent activity against *Pneumocystis carinii* and *Nocardia* species.
1.2.8 **Oxazolidinones**

Oxazolidinones are organic and contain a ring of 2-Oxazolidone with oxygen and nitrogen. Linezolid was the first antibiotic in this new class, and it exerts its effect by binding to a ribosome sub-unit, inhibiting protein synthesis in Gram positive bacteria. The elimination of the drug is predominantly renal. It has a bactericidal effect against most *Streptococcus spp* and *Enterococcus spp*, and it has a bacteriostatic effect against *Staphylococcus spp*. Linezolid is mainly prescribed for MRSA infections or other multiresistant bacteria as an alternative to the glycopeptide agent Vancomycin.

1.2.9 **Amphotericin B**

Amphotericin B is derived from *Streptomyces nodosus* and its name is derived from its amphoteric properties. The mechanism of action is through association of amphotericin to fungal membrane ergosterols, which causes leakage of potassium and intracellular components leading to cell death. Higher doses are fungicidal and lower doses are fungistatic. Amphotericin is used in the treatment of systemic fungal infections in immunocompromised patients. The agent is also active in candidiasis, aspergillosis, cryptococcal meningitis and visceral leishmaniasis. It is also used empirically in the treatment of fever in neutropenic patients that do not respond to broad-spectrum antibiotics.

1.2.10 **Tetracyclines**

Tetracyclines are a group of broad – spectrum antibiotics whose general usefulness has been reduced with the onset of bacterial resistance. Despite this, they remain the treatment of choice for some specific indications. Tetracyclines are generally used in the treatment of infections of the urinary tract and the intestines, and are used in the treatment of chlamydia, especially in patients allergic to β – lactams and macrolides. Tetracycline inhibits cell growth by inhibiting translation. It binds to the 16S part of the 30S ribosomal subunit and prevents the amino-acyl tRNA from binding to the A site of the ribosome. The binding is reversible in nature. Their most common current use is in the treatment of moderately severe acne and rosacea (tetracycline, oxytetracycline, doxycycline, or minocycline). Doxycycline is also used as a prophylactic treatment for infection by *Bacillus anthracis* (anthrax) and is effective against Yersinia pestis, the infectious agent...
of bubonic plague. It is also used for malaria treatment and prophylaxis, as well as treating elephantiasis. Tetracyclines remain the treatment of choice for infections caused by Chlamydia, Rickettsia, Brucellosis, and Spirochetal infections. In addition, they may be used to treat anthrax, plague, tularemia, and Legionnaires' disease. It is also used in veterinary medicine on pigs and alike. They may have a role in reducing the duration and severity of cholera, although drug-resistance is occurring, and their effects on overall mortality is questioned. Tetracycline derivatives are currently being investigated for the treatment of certain inflammatory disorders

1.3 Mechanisms of Action of Antimicrobials: Antibiotics

Antibiotics can be classified in several ways. One common method of classification is by their mechanism of action against the infecting bacteria. Some antibiotics act by interfering with the synthesis of proteins and nucleic acids in the bacteria, while others attack the cell wall or disrupt the cell membrane [24]. A clinically important group of antibiotics interferes with the synthesis of the peptidoglycan, the most important component of the cell wall. This group of antibiotics is called the β-lactams and can further be divided into the penicillins, cephalosporins, monobactams and carbapenems [24]. Another large group of antibiotics inhibits the synthesis of various intracellular molecules, such as DNA, RNA, ribosomes and proteins. Examples of such antibiotics are rifampicin, which inhibits the RNA polymerase, and the quinolones [25], which inhibit the enzymes responsible for coiling and uncoiling the DNA molecule, a process necessary for DNA replication and transcription. There are also other mechanisms viz; macrolides interfere with the 50S subunit of the ribosome, whereas tetracyclines affect the 30S ribosomal subunit, both inhibiting protein synthesis (Table 1).

Antibiotics may also be classified as bactericidal or bacteriostatic [27]. In general, antibiotics attacking the cell wall belong to the group of bactericidal drugs, since a defective cell wall eventually will cause the bacteria to lyse and die. Among the bacteriostatic antibiotics, macrolides, lincosamides and chloramphenicol can be mentioned. With bacteriostatic drugs, the host immune system plays an important role, helping to clear the infection once bacterial growth has subsided. For that reason, bactericidal drugs should be considered in patients that are immuno-compromised, as
well as in patients with serious infections such as endocarditis and meningitis, in which cases a fast reduction of bacteria is warranted [28].

### Table 1: Mechanisms of action of different antibiotic classes [26]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Antibiotic Class</th>
<th>Molecular target</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>β – lactams</td>
<td>Cell wall, Penicillin binding proteins</td>
<td>Benzylpenicillin, Ampicillin Amoxycillin, Cephalosporins Carbapenems, Monobactams</td>
</tr>
<tr>
<td>2</td>
<td>Aminoglycosides</td>
<td>30S, 50S ribosomal subunits</td>
<td>Gentamicin, Tobramycin, Amikacin</td>
</tr>
<tr>
<td>3</td>
<td>Trimethoprim, Sulphonamides</td>
<td>Folate synthesis</td>
<td>Trimethoprim, Sulfadiazine</td>
</tr>
<tr>
<td>4</td>
<td>Quinolones</td>
<td>Gyrase, Topoisomerase IV</td>
<td>Nalidixic acid, Fluoroquinolones, Erythromycin, Clarithromycin</td>
</tr>
<tr>
<td>5</td>
<td>Macrolides</td>
<td>50S ribosomal subunit</td>
<td>Erythromycin, Clarithromycin</td>
</tr>
<tr>
<td>6</td>
<td>Lincosamides</td>
<td>50S ribosomal subunit</td>
<td>Clindamycin</td>
</tr>
<tr>
<td>7</td>
<td>Streptogramins</td>
<td>50S ribosomal subunit</td>
<td>Quinopristin/Dalfopristin</td>
</tr>
<tr>
<td>8</td>
<td>Tetracycline</td>
<td>30S ribosomal subunit</td>
<td>Tetracycline, Doxycycline</td>
</tr>
<tr>
<td>9</td>
<td>Glycopeptides</td>
<td>Cell wall peptidoglycan</td>
<td>Vancomycin, Teicoplanin</td>
</tr>
<tr>
<td>10</td>
<td>Chloramphenicol</td>
<td>50S ribosomal subunits</td>
<td>Chloramphenicol</td>
</tr>
<tr>
<td>11</td>
<td>Rifamycins</td>
<td>RNA polymerase</td>
<td>Rifampicin</td>
</tr>
<tr>
<td>11</td>
<td>Polymyxin</td>
<td>Cell membrane</td>
<td>Polymyxin B, Colistin</td>
</tr>
<tr>
<td>12</td>
<td>Oxazolidinone</td>
<td>50S ribosomal subunit</td>
<td>Linezolid</td>
</tr>
</tbody>
</table>

### 1.4 Antimicrobial Resistance

For many years antibiotics seems to be winning the war against infectious disease. However, despite the successful development of several different antibiotic classes, the introduction of a new drug was almost always followed by resistance. Shortly after the introduction of penicillin, resistance was detected in Staphylococcus aureus, and by 1970
most *S. aureus* isolates were penicillin-resistant [29]. In a similar manner, clinicians soon witnessed clinical failure of other antibiotics due to bacterial resistance development. For every decade to follow, bacteria resistant not only to single but multiple antibiotics have become more and more widespread [30]. Today, we are facing a problem of multi-resistant *Salmonella, Shigella, Campylobacter, Pseudomonas aeruginosa* and *Mycobacterium tuberculosis, penicillin-resistant pneumococci, vancomycin-resistant enterococci* and methicillin- and vancomycin resistant *Staphylococcus aureus* [31-33]. At the same time fewer antibiotics are being produced, and it is becoming more and more apparent that a careful and prudent use of antibiotics is necessary in order to curtail the development of bacterial resistance [34].

1.5 **Mechanisms of Antimicrobial Resistance**

In basic terms, the increase in prevalence of antibiotic resistant bacteria during the last decades can be attributed to evolution and natural selection. All populations of organisms, including bacteria, will include variants with unusual traits, in this case the ability to withstand antibiotics. Consequently, every time a specific antibiotic is used, the antibiotic resistance trait will be positively selected, and the bacteria carrying this trait will increase in number and eventually pre-dominate the population.

The bacterial traits of antibiotic resistance may be due to several different Mechanisms [35], including:

- A decreased uptake of the drug
- An increased export of the drug
- Inactivation or modification of the drug target
- The introduction of a new drug resistant target
- Hydrolysis of the antibiotic
- Modification of the drug
- Prevention of activation of the drug.

Antibiotic resistance traits are naturally occurring in the environment and have been so since long before antibiotics were introduced into human medicine [36]. One theory for the presence of antimicrobial resistance genes in the environment is that they originate
from bacteria or fungi that use them as protection from antibiotics produced by other bacteria \[37\]. Another theory for the source of antibiotic resistance determinants is that certain housekeeping genes, such as sugar kinases and acetyltransferases, may have evolved to modify antibiotics, as in the case of aminoglycoside resistance \[36\]. Second, some bacteria are naturally resistant to certain antibiotics on account of their genetic composition. For example, *Mycoplasma spp.* is always resistant to lactams, since this species lacks the peptidoglycan in the cell wall. Bacteria carrying such resistant traits are designated to be intrinsically resistant, i.e. naturally resistant to an antibiotic without any genetic alterations. Finally, bacteria may be genetically altered to become resistant, a process called acquired resistance \[35\].

Bacteria can acquire resistance by either of two mechanisms;
- Spontaneous mutation
- Horizontal transfer.

Mutations may render the bacteria resistant by modifying the drug target (mutations in ribosomal proteins, penicillin-binding proteins etc.), by changing the uptake of the drug (mutations in a porin) or by inducing an increased efflux of the drug (mutations causing overexpression of efflux pumps) \[35, 38\]. Spontaneous mutation is dependent on the mutation rate and the presence of proofreading and repair mechanisms \[39\]. Some strains display an extremely high mutation rate and are called mutator strains. These bacteria are usually defective in the mismatch repair system or lack the ability of proofreading \[37, 40\]. The role that mutator strains might play in generating and speeding up antibiotic resistance development is discussed further in the (Table 2) “Rate of formation of resistant mutants”.

Horizontal transfer is a mechanism that allows bacteria to share genetic material and thereby maintain genetic diversity \[42\]. It also constitutes the main mechanism for acquiring antibiotic resistance determinants. Essentially, three different processes are involved in horizontal gene transfer: conjugation, transduction and transformation \[43\].
Table 2: Major bacterial pathogens and resistance patterns [41]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Bacterial pathogen</th>
<th>Antibiotic resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E. Coli</td>
<td>β-lactam resistance due to plasmid-mediated lactamases, trimethoprim resistance, quinolone resistance due to mutation in gyrA</td>
</tr>
<tr>
<td>2</td>
<td>Klebsiella</td>
<td>β-lactam resistance due to plasmid-mediated lactamases</td>
</tr>
<tr>
<td>3</td>
<td>Helicobacter pylori</td>
<td>Macrolide and metronidazole resistance</td>
</tr>
<tr>
<td>4</td>
<td>Enterococci</td>
<td>Glycopeptide resistance, aminoglycoside resistance, quinolone resistance, penicillin/carbapenem resistance (mainly E. faecium)</td>
</tr>
<tr>
<td>5</td>
<td>Staphylococcus aureus</td>
<td>Methicillin resistance (MRSA) often combined with multidrug resistance (aminoglycosides, macrolides, tetracyclines)</td>
</tr>
<tr>
<td>6</td>
<td>Streptococcus pneumoniae</td>
<td>Penicillin resistance combined with multidrug resistance (tetracycline, macrolides, chloramphenicol) Macrolide resistance, tetracycline resistance</td>
</tr>
<tr>
<td>7</td>
<td>Haemophilus influenza</td>
<td>β-lactam resistance mediated by β-lactamases or target modification</td>
</tr>
<tr>
<td>8</td>
<td>Mycobacteria</td>
<td>Multidrug resistance (isoniazid, rifampicin and others)</td>
</tr>
</tbody>
</table>

Since these mechanisms can occur not only within the same but also within different species, horizontal transfer constitutes a major force behind the spread of resistance [42, 44]. The manner by which horizontal transfer renders bacteria resistant is primarily via the introduction of new antibiotic targets. This is commonly seen as the recruitment of new genes carried on plasmids or transposons. Thus, as opposed to spontaneous mutation, the resistance determinant is pre-existing in a reservoir and is not the direct result of antibiotic selection of mutants from within an entirely susceptible bacterial population.
A new antibiotic target could also be introduced by transformation of DNA and a subsequent recombination into the chromosome. An example of a resistance determinant that has originated from horizontal transfer and transformation is the development of mosaic genes of penicillin binding proteins in *S. pneumoniae*, conferring penicillin resistance [45]. Few human pathogens have this ability; most other clinically important pathogens become penicillin-resistant due to the acquisition of genes encoding β-lactamases, which inactivate the β-lactams.

### 1.6 Tuberculosis: Disease Profile

Tuberculosis (TB) is caused by the bacterium *Mycobacterium tuberculosis* (MTB). Infection normally occurs through inhaling bacteria-containing droplets emitted from infected individuals when coughing, sneezing or even speaking. Inhaled bacteria are phagocytosed by macrophages in the lungs. To combat the infection, these macrophages aggregate, and recruit various T-cells and granulocytes to form granuloma, so-called tubercles. The bacterium can lie dormant in this granuloma for decades [46, 47]. Actually, only approximately 10% of all MTB infections ever give rise to the disease in the infected individual. Triggering of TB is associated with collapse of the tubercles and resumed bacterial replication. In situations where the immune system is weakened, as in individuals infected with HIV, the lifetime risk of developing TB for an infected individual increases to 50% [48].

Pulmonary TB is the most common form of the disease in non-HIV infected patients. In conjunction with HIV, MTB spreads more readily to other parts of the body, and non-pulmonary disease is as common as the pulmonary form. The non-pulmonary forms include infections of the meninges and the central nervous system, the genitourinary system and the lymphatic system, as well as a disseminated form. The bacteria cause necrosis and tissue remodelling in the infected organs, with clinical effect depending on the site of action. In the lungs, TB initially causes intense coughing and sometimes pneumothorax with associated pain, and eventually dyspnoea and severe respiratory failure. Systemic symptoms include weight loss and fever [49].
In 2008, an estimated 390,000-510,000 cases of MDR-TB emerged globally (best estimate 440,000 cases). Among all incident TB cases globally, 3.6% (95% confidence interval (CI): 3.0–4.4) are estimated to have MDR-TB. These estimates, which lie in the same range as the previous ones, are based on more data and a revised methodology. Almost 50% of MDR-TB cases worldwide are estimated to occur in China and India. In 2008, MDR-TB caused an estimated 150,000 deaths.

The MTB bacterium was discovered as the causative agent in TB by Robert Koch in 1882. For this and other findings in the field of microbiology, he received the Nobel Prize in 1905. The MTB bacterium has evolved to survive within macrophages, and is characterised, together with other mycobacteria, by extraordinary slow growth and a thick, complex cell wall with a unique composition. The complete genome of MTB was published in 1998, and is currently believed to contain 3995 genes, of which 376 encode proteins with no homology to known proteins. The thick cell wall of MTB makes it immune to most common anti-bacterial chemotherapies. There are currently only five first-line anti-tuberculosis drugs: isoniazid, rifampicin, pyrazinamide, ethambutol and streptomycin. These are usually given in combination. Isoniazid was discovered in 1952, and is an irreversible enzyme inhibitor, acting on the cell wall synthesis of MTB. It is still the most effective TB drug, and is included in all recommended first-line treatments. Ethambutol is another cell wall synthesis inhibitor, rifampicin or reifampin inhibits transcription, streptomycin acts on the ribosome to inhibit translation and pyrazinamide inhibits the transport of nutrients over the bacterial cell membrane. Even though there are a number of less effective second-line antibiotics, development of multidrug-resistant strains of MTB underlines the need for effective novel drugs to treat TB.

1.6.1 A History of Tuberculosis Chemotherapy

The chemotherapy of infectious diseases, using sulfonamide and penicillins, had been underway for several years, but these molecules were ineffective against Mycobacterium tuberculosis. Since 1914, Selman A. Waksman had been systematically screening soil bacteria and fungi. In 1939 had discovered the marked inhibitory effect of certain fungi, especially actinomycetes, on bacterial growth. In 1940, he and his team at Rutgers...
University in New Jersey were able to isolate an effective anti-TB antibiotic, actinomycin; however, this proved to be too toxic for use in humans or animals. Success came in 1943. In test animals, streptomycin, purified from *Streptomyces griseus*, combined maximal inhibition of *M. tuberculosis* with relatively low toxicity. On November 20, 1944, the antibiotic was administered for the first time to a critically ill TB patient. The effect was almost immediately impressive. His advanced disease was visibly arrested, the bacteria disappeared from his sputum, and he made a rapid recovery. However, the new drug had side effects especially on the inner ear but the fact remained that, *M. tuberculosis* was no longer a bacteriological exception; it could be assailed and beaten into retreat within the human body.

A rapid succession of anti-TB drugs appeared in the following years. These were important because with streptomycin monotherapy (one drug treatment), resistant mutants began to appear with a few months, endangering the success of antibiotic therapy. However, it was soon demonstrated that this problem could be overcome with the combination of two or three drugs and most of mentioned in (Fig. 2).

### 1.6.2 Current Tuberculosis Drug Therapy

Most of the drugs which composed the arsenal of the first-line TB treatment were discovered during the 1950’s and the 60’s (Fig. 2). In 1944, streptomycin was the first compound used to treat TB [61]. This amino glycoside interferes with protein biosynthesis through an interaction with the small 30S subunit of the ribosome [62, 63]. The discovery of para-aminosalicylic acid in 1946 was quickly followed by the important identification of isoniazid (INH) [64], one of the most active anti-TB drugs used today. Mechanisms of action for both compounds were unknown during their development, and the target of para-aminosalicylic acid is still the subject of investigations [65]. Inhibition of mycolic acids biosynthesis, one of the essential components of the mycobacterial cell wall was determined as isoniazid mechanism of action [66, 67]. Pyrazinamide (PZA) appeared as a potential TB drug in 1952 [68]. Its introduction in the TB treatment in the 1980s was a great success as it allowed to shorten the duration of the TB therapy from 9 to 6 months. Despite an important similarity of structures between isoniazid and pyrazinamide, their mechanisms of action are totally different. Pyrazinamide activity is
dependent on pyrazinoic acid release, which causes intake of proton and dysfunction of the pH balance of mycobacteria [69, 70]. It has been recently shown that pyrazinoic acid targets the ribosomal protein S1, an essential protein involved in the ribosome-sparing process of transtranslation [71].

Ethambutol (EMB) and rifampicin (RIF), the two last derivatives used in the TB first-line treatment, were discovered during the 60’s. Ethambutol is an ethylenediamine discovered in 1961 [72], which affects the cell wall by specifically targeting the polymerization of arabinogalactane and lipoarabinomannane [73]. Finally, rifampicin appeared as a drug of choice for TB treatment [74, 75] around 1970, by acting on replicating and non-replicating mycobacteria. This derivative belongs to the rifamycine family and inhibits bacterial RNA synthesis by binding to the b-subunit of the DNA-dependent polymerase [76].

![Fig. 2 Anti-tubercular Agents](image_url)

Despite the efficiency of the drugs alone, a significant improvement of the treatment was obtained with combined therapy in order to limit the apparition of resistant strains. Today, the current standard regimen (DOTS) for TB recommended by WHO is a combination of isoniazid, rifampin, ethambutol and pyrazinamide for a 6 months therapy.
To treat MDR-TB, WHO recommends the use of second-line drugs which include aminoglycosides (kanamycin, amikacin), capreomycin, cycloserin, para-aminosalicylic acid, thioamides (ethionamide (ETH), prothionamide), and fluoroquinolones (ciprofloxacin, ofloxacin, levofloxacin). Ethionamide and prothionamide are isoniazid analogues discovered in 1956 [77]. As isoniazid, they target mycolic acids biosynthesis through the inhibition of InhA [78]. Isoniazid and the two thioamides analogues are prodrugs that respectively require bioactivation by two distinct proteins: KatG and EthA [79-81]. D-cycloserine, another cell wall inhibitor, was discovered in 1969 [82]. This compound triggers peptidoglycan synthesis through D-alanine racemase and D-alanine ligase inhibition [83, 84]. In the mean time, aminoglycoside analogues (kanamycin, amikacin) [85] and cyclic peptide analogues (viomycin, capreomycin) of streptomycin were reported. These compounds target the small subunit 30S of the ribosome. The most recent anti-TB drugs are fluoroquinolones that derived from nalidixic acid, discovered in the early 60’s for its antibacterial activity [86]. Among all the antibacterial fluoroquinolones developed, ciprofloxacin, ofloxacin and levofloxacin are the most widely used in anti-TB treatments [87]. In mycobacteria, fluoroquinolones target the DNA gyrase, which is the sole type II topoisomerase in this organism [88-91].

1.6.3 Limitation of Current drug therapy and need for new drug targets
Despite all the advances in the chemotherapy of tuberculosis, the treatment of the disease still continues to be one of the most important and challenging problems in the field of chemotherapy. The following factors render the management of this disease a complex and protracted procedure.

- Inadequate defense mechanism of the host.
- Metabolic character of the tubercle bacillus.
- Rapid development of resistance by *M. tuberculosis* to almost all of available antitubercular drugs, limiting the ultimate value of these drugs.
- Persistence of dormant but viable tubercle bacilli in the tissues of the majority of patient inspite of extensive chemotherapy.
- Lack of bactericidal action of mast of the anti-tubercular drugs.
✓ Morbid toxicity of many of the drugs, which prevents them from being administered in therapeutic doses.

✓ The economic condition of the underdeveloped countries does not permit the use of many of the drugs due to their high cost and longer duration of treatment required to eradicate mycobacterium. Considering the above factors, it is pertinent to mention here that a drug, which can effectively eradicate tubercle bacilli within a short duration of time, is most desirable and progress in this area is the need of the hour.

1.6.4 Antitubercular Drugs in Pipeline and clinical trials

![Chemical structures of various antitubercular drugs](image)

Fig. 3 Current Drugs Discovery for Tuberculosis
Drug discovery for Tuberculosis have been made over the last 10 years, with TB drugs that have entered clinical trials (Fig. 3). Fluoroquinolones gatifloxacin and moxifloxacin (Phase 3), oxazolidinone linezolid and nitroimidazole metronidazole (Phase 2) have been repurposed for tuberculosis. New chemical entities have also progressed in clinical development based on optimization of known chemical scaffolds: this is the case for nitroimidazole derivatives OPC-67683 and PA-824 both in phase 2, substituted ethylenediamine SQ109 (phase 2), oxazolidinone analogues PNU-100480 (phase 2) and AZD5847 (phase 1). Finally, promising diarylquinoline TMC207, first compound of a new class of antituberculosis drugs is currently evaluated in Phase 2.

1.6.4.1 Compounds in phase 3 clinical trials

The fluoroquinolones gatifloxacin and moxifloxacin were marketed in 1999 for the treatment of respiratory tract infections. These two molecules are currently in phase 3 clinical trials for the treatment of TB [93]. *M. tuberculosis* does not possess any type IV topoisomerase. Thus, fluoroquinolones target specifically the mycobacterial topoisomerase II DNA gyrase. This implies that large spectra fluoroquinolones suffer from suboptimal inhibition of topoisomerase II and thus can be improved [94]. Gatifloxacin and moxifloxacin have better in vitro activity against *M. tuberculosis* than the older fluoroquinolones ciprofloxacin and ofloxacin [95]. Gatifloxacin and moxifloxacin are currently being evaluated in a phase 3 clinical trials. The aim of these studies is to evaluate the efficacy and safety of gatifloxacin and moxifloxacin and the possibility of reducing the duration of tuberculosis therapy from six to four months [96, 97].

1.6.4.2 Compounds in phase 2 clinical trials

1) Diarylquinoline: TMC207

TMC207 (R207910, Bedaquiline) is a novel diarylquinoline belonging to a new class of anti-tuberculosis drugs. This compound was discovered by Johnson & Johnson through a whole-cell screening on *Mycobacterium smegmatis* and it is currently clinically developed by Tibotec in collaboration with the TB alliance. TMC207 inhibits the proton transfer chain of the mycobacterial ATP synthase [98]. The mechanism of action of the diarylquinoline was originally proposed after isolation of mutant strains of *M. tuberculosis* and *M. smegmatis* that were resistant to TMC207. Their genomes were
sequenced and compared to susceptible strains [99]. The only common mutation was localized in the atpE gene encoding for subunit c of ATP synthase which was further validated as the compound’s precise target [100]. TMC207 is not active on human mitochondrial ATP synthase [101]. Whereas derived from quinolones, TMC207 has no inhibitory effect on the DNA gyrase of *M. tuberculosis*.

2) Nitroimidazoles

   i) Metronidazole  
   Metronidazole developed in the 1960’s, is currently marketed to treat protozoa and anaerobic bacteria infections. Because of its preferential activity against anaerobic organisms [102, 103], metronidazole was considered as an attractive compound to treat non replicating *M. tuberculosis*. In 1999 Brooks *et al.* demonstrated that metronidazole exhibited only modest activity on *in vitro* and *in vivo* models, alone or combined with isoniazid [104]. Currently, a phase 2 study driven by the National Institute of Allergy and Infectious Diseases (NIAID) in South Korea is exploring the impact of adding metronidazole to the standard second-line drug regimen [105].

   ii) PA-824  
   Initially, bicyclic nitroimidazofurans were investigated as potential radiosensitizing agents for use in cancer radiotherapy [106] but were also found to display some antitubercular activities in both *in vitro* and *in vivo* models [107, 108]. Very promising results were obtained for the lead compound of this chemical series, Nevertheless, the strong activities obtained with these compounds suggested that the bicyclic nitroimidazole moiety might be an interesting pharmacophore. Based on this observation, a chemical library of 328 nitroimidazopyrans [109, 110] was designed and evaluated on *M. tuberculosis*. One member, PA-824, was identified as a promising antitubercular agent. PA-824 is active against *M. tuberculosis* in both aerobic and hypoxic conditions [110]. Both activities are supposed to be mediated by different mechanisms of actions. Stover *et al.* [110] showed that PA-824 inhibits the biosynthesis of mycolic acids in a dose-dependent manner.

   iii) Diamine derivative: SQ109  
   SQ109 is a synthetic analogue of ethambutol [111]. It was discovered from a focused library of ethylenediamine analogues containing 63,238 compounds [112]. The exact
mechanism of action of SQ109 is not yet known. SQ109, as ethambutol, was shown to over-produce the ATP-dependent DNA/RNA helicase and to reduce the production of the β-ketoacyl-acyl carrier protein synthase which may explain its action on mycobacterial cell wall synthesis \[113\]. Interestingly, SQ109 is still active against EMB-resistant strains, therefore SQ109 is believed to act in a different manner than ethambutol \[111\]. It was also shown that SQ109 is active against RIF-resistant strains \[114\].

iv) Oxazolidinones: Linezolid
Linezolid was introduced in the USA for the treatment of patients with infections caused by Gram-positive pathogens (Staphylococci, Streptococci and Enterococci) responsible for skin and soft tissue infections, pneumonias and bacteraemias \[115\]. Linezolid is used for courses of treatment up to 28 days. This compound belongs to the (S)-oxazolidin-2-one family class of compounds and is a direct analogue of DuP105 and DuP721 described to have MIC of 0.3-1.25 µg/mL against \textit{M. tuberculosis} \[116\] and which development was discontinued in Phase 1 due to toxicity issues. Linezolid presents a unique mechanism of action which was supported by the lack of cross-resistance between oxazolidinones and other antibiotics. It binds to the 23S RNA in the 50S ribosomal subunit and limits the growth of bacteria by disrupting its production of proteins in the first step of the synthesis by inhibiting formation of the initiation complex \[117\].

v) PNU-100480
As discussed previously, the use of linezolid is limited by adverse effects that occur with long-term administrations. Therefore, new analogues showing identical or better in vivo activities and a better therapeutic index would be useful. The development of PNU-100480 (Sutezolid), a close structural analogue of linezolid was initiated by Upjohn.

1.6.4.3 Compound in phase 1 clinical trials
1) Oxazolidinone AZD5847
New oxazolidinone derivative, AZD5847 (also known as AZD2563) currently in phase 1 clinical trials, is developed by AstraZeneca. Two studies, a single ascending dose and multiple ascending dose over 14 days, have now been completed for AZD5847 \[118\]. Bioavailability in fasted volunteers was reported to decrease with increasing doses, declining from 100% at 50 mg to less than 30% at 1200 mg. However, this tendency was corrected by food intake. AZD5847 was tolerated over 14 days in healthy volunteers. The
doses selected for investigation in phase 2 studies are 500 mg once and twice daily, 800 mg twice daily and 1200 mg once daily and will be compared to Rifafour (RIF/ INH/ PZA/ EMB) 1 pill per or once daily [119]. A phase 2a is scheduled to start in 2012.

1.6.4.4 Compounds in preclinical development

1) Fluoroquinolone: DC-159a
As moxifloxacin and gatifloxacin, DC-159a belongs to a new generation of fluoroquinolones. The mechanism of action of DC-159a is still under investigation, but as other quinolone derivatives, DC-159a probably affects GyrA activity, which plays important roles in DNA replication [120, 121]. However DC-159a resistant mutants revealed patterns of mutations in GyrA different than the ones observed in quinolones-resistant strains [122]. DC-159a showed better in vitro and in vivo activities against quinolone resistant multidrug resistant tuberculosis strains (QR-MDR-TB) than some other fluoroquinolones [122]. Therefore, it has been proposed that DC-159a may be a replacement drug for the treatment of QR-MDR cases.

2) Diamine derivative: SQ609
While SQ109 was under development, several new dipiperidine analogues were designed and they demonstrated activity against M. tuberculosis [123]. The solid-phase synthesis and screening of a focused library of 10,358 diamines led to the discovery of dipiperidine analogue SQ609. In M. tuberculosis infected macrophages in vitro, a concentration of 4 µg/mL of SQ609 was able to inhibit 90% of bacterial growth without showing any toxic effect. In vivo efficacy of SQ609 was evaluated in M. tuberculosis H37Rv infected mice. C3H/He mice intravenously infected were treated once daily with SQ609 at 10 mg/kg for 2 weeks. SQ609 was shown to prevent weight loss of the animals and was able to prolong therapeutic effect 2 weeks after the end of the treatment [124]. SQ609 is currently being evaluated in preclinical studies.

3) Nitrophenyl derivatives: BTZ043
A new class of sulphur containing heterocyclic called benzothiazinones (BTZ) has been recently described as potent antimycobacterial agents [125]. The structure-activity relationships study showed that sulphur atom and one or two nitro groups on the aromatic structure were required to inhibit bacterial growth in vitro. Compound BTZ038, was the most active of the series.
4) DNB1
Dinitrobenzamide analogues as DNB1 were recently identified thanks to a phenotypic cell-based assay that uses automated confocal fluorescence microscopy [126, 127]. They showed high activity against sensitive and XDR M. tuberculosis strains. These derivatives were also shown to inhibit decaprenylphospho-arabinose synthesis by targeting decaprenylphosphoribose 2 epimerase DprE1 and are currently under development.

5) 1,2,4-Oxadiazole derivative: BDM31343
Many antituberculous agents are prodrugs. Among them, the second-line drug ethionamide is activated by a mycobacterial mono-oxygenase called EthA. The expression of ethA and thus the potency of the drug are limited by the transcriptional repressor EthR [128]. In vitro overproduction of EthR was shown to confer resistance to ethionamide whereas EthA overproduction via ethR KO conferred at least a 25-fold increase of ethionamide potency [129]. A new concept emerged from this observation and drug-like inhibitors of EthR were designed and validated as boosters of ethionamide activity in order to improve therapeutic index of this antibiotic [130, 131]. Hit compound BDM14500 was identified thanks to a functional screening of a focused library. BDM14500 was validated in vitro for its capacity to boost the antimycobacterial activity of ethionamide. BDM14500 was in the same time cocry stallized with EthR and revealed to occupy as expected the ligand binding domain of the protein. Optimization process led to the discovery of BDM31343 that proved to be 10-fold more active, more soluble in aqueous solution and more stable in mouse liver microsomes [131].

For the 1st time in 40 years, a large number of consortia and pharmaceutical companies have exhibited massive drug discovery efforts to develop new chemical series using either target-based or phenotypic screens. There are at least ten compounds in clinical trials and strategies for the development of new molecules are ready to fuel the pipeline. Most of them are still in preclinical testing but one might expect a candidate to rapidly reach the clinic. New targets have been identified and validated with drug-like molecules and the most advanced compound TMC207 might open a bright avenue for the TB treatment. Number of pharmaceutical companies involved in TB drug development projects has also increased. However, drug development is a long process especially for
TB and it is likely that only one or two new drugs will arrive on the market from these efforts. Bacterial resistance and thus requirement for combinations of molecules tend to suggest that the current development pipeline is not yet sufficiently backed-up to overcome the major unmet medical needs in TB treatment. Efforts are still eagerly needed if we want to have soon a chance to win the battle against this millenary scourge.

1.7 Malaria: Disease Profile

Malaria is one of the earliest known diseases. The name originates from the Italian “mala aria”, which means bad air; a suitable name since it was thought to arise from exhalation of swamps. The true cause of the disease became clear first in 1880, when the French researcher Laveran (Nobel Prize in Medicine 1907) discovered the malaria parasite in human blood [132]. Some years later (1897) [133], the English physician Ross (Nobel Prize in Medicine 1902) and the Italian zoologist Grassi (1898) [134] demonstrated that the parasite was injected into the human bloodstream through the bite of an infected female mosquito [135].

Malaria parasites belong to the protozoan subkingdom of the class Sporozoa. Four species of the Plasmodium genus are responsible for human malaria: *P. vivax*, *P. ovale*, *P. malariae*, and *P. falciparum* [136]. On an evolutionary basis *P. vivax*, *P. ovale*, and *P. malariae* are closely related to the simian malarials, whereas *P. falciparum* is thought to be of a more recent origin closely related to the malarials of birds [137]. The natural vectors of the malaria parasites are female Anopheles mosquitoes. Of the approximately 400 species about 60 transmit malaria under natural conditions [138].

The clinical picture of malaria varies with each species. However, the usual symptoms are chills and fever at more or less pronounced intervals [136]. Due to development of so called severe malaria [139], infection caused by *P. falciparum* is the only one normally lethal. Severe malaria is a complex multisystem disorder involving adherence of parasites to blood vessel endothelial cells and severe anemia [139, 140].

1.7.1 Parasite life cycle

Malaria parasite development requires mosquitoes and human hosts to complete its life cycle. The parasite undergoes several developmental stages on its way to cause disease in humans. During its two-host life cycle, *P. falciparum* undergoes ten morphological transitions in five different host tissues [141].
The cycle in humans includes:

- **The pre-erythrocytic stage**, which is the first stage of infection in humans where sporozoites are inoculated to infect the hepatocytes.

- **The erythrocytic stage**, which is the asexual reproduction of the parasite in the blood that causes the clinical symptoms of the disease.

- **The gametocyte stage**, which enables the sexual reproduction of the parasites in the mosquito and further transmission (Fig. 4).

The pre-erythrocytic stage begins when an infected female Anopheles mosquito inoculates sporozoites into the skin or into the bloodstream of humans during a blood meal. Sporozoites circulate transiently in the bloodstream before invading hepatocytes, where an asexual cycle occurs. It is estimated that mosquitoes transmit fewer than 100 sporozoites per bite [142, 143]. Recent studies have shown that sporozoites can remain for up to 6 hours at the site of injection [144], and that one-third of those leaving the injection site may enter the draining lymph nodes via the lymphatic vessels [145]. The capacity of each of these sporozoites to yield an asexual erythrocytic-stage infection is low. In humans, at least bites of five *P. falciparum* infected mosquitoes are necessary to assure that 100% of the volunteers get infected [146]. When sporozoites reach the liver parenchyma, they continue to migrate through several hepatocytes before they finally infect one. This migration seems to be beneficial for malaria infections in at least two different ways: by activating sporozoites for infection and by increasing the susceptibility of host hepatocytes [147]. After a week, the rupture of merosomes within the lung microvasculature [148] releases thousands of infectious merozoites into the bloodstream, where they invade circulating erythrocytes and initiate the clinically important intraerythrocytic cycle of the asexual replication during the following 48 hours. This stage is responsible for all the clinical symptoms associated with the disease. After 24-32 hours, when young parasites mature from rings to the trophozoite stages, infected erythrocytes adhere to endothelial cells in the microcirculation of various organs (sequestration) causing cerebral malaria if sequestrated in the brain. The trophozoites mature into schizonts, which finally rupture and release 16-32 daughter merozoites that invade fresh red-blood cells to perpetuate the asexual life cycle. As a survival strategy, blood-stage parasites have been shown to infect, survive, and replicate within CD317+ dendritic cells.
(DCs) and small numbers of these cells release parasites infectious for erythrocytes in vivo in a murine model [149].

Some of the parasites inside erythrocytes differentiate into male and female gametocytes. It remains poorly understood which factors stimulate gametogenesis [150]. However, environmental factors, drugs and innate immune factors have been reported to influence gametogenesis [151, 152]. Without treatment, most patients with P. falciparum malaria will develop gametocytemia within 10 to 40 days after the onset of the parasitemia [153].
Upon ingestion by a feeding female mosquito, the male and female gametes undergo fertilization in the mosquito midgut to form a zygote and subsequently a motile ookinete. The ookinetes penetrate the midgut epithelial cells and rest between the midgut epithelial cells and the basal lamina to form oocysts. The oocysts undergo a complex asexual development phase, which eventually generates infective sporozoites that can be introduced into the human host at the next blood meal, thereby perpetuating the continuation of the parasite life cycle [154].

1.7.2 Chemotherapy for Malaria

Chemotherapy has traditionally played an important role in the treatment and control of malaria. Quinoline containing antimalarial components is the most effective drugs for malaria chemotherapy. This group of compounds has evolved from the structural modification of quinine and includes 4-aminoquinoline compounds such as chloroquine and mefloquine of which former is more effective, cheap, safe and commonly available drug.

The dihydrofolate reductase inhibitors include proguanil, chlorproguanil, pyrimethamine and trimethoprim and sulfa drugs like dapsone, sulfalene, sulfamethoxazole and sulfadoxine. These drugs are used in combinations. The classical such combination is sulphadoxine and pyrimethamine (SP) used as first line drug in Thailand and other parts of the world. Tetracycline and its derivatives such as doxycycline are very potent antimalarials and are used for both treatment and prophylaxis. In areas where response to quinine has deteriorated, tetracyclines are often used in combination with quinine to improve cure rates.

The other useful antimalarials are Artemisinin compounds synthesised from the plant Artemisia annua. These compounds (artesunate, artemether, arteether) are most effective antimalarials and seem to have effect on protein synthesis by the malaria parasite. These are used for the treatment of severe malaria and have shown very rapid parasite clearance in comparison to quinine compounds. Artemisinin and mefloquine combination is being used in some Southeast Asian countries, for the treatment of uncomplicated malaria, where the multi drug resistant strains of P. falciparum are prevalent [155].
1.7.2.1 Quinoline-Methanols

This class of agents originates from the cinchona bark alkaloids. The two major agents are quinine and mefloquine. For hundreds of years quinine was the only known effective treatment for malaria [156]. Today, the advent of drug resistance has made its importance return mainly for the treatment of severe malaria. Mefloquine is a relatively expensive drug commonly used as a prophylactic for travelers to chloroquine-resistant areas. The mechanism of action of this group has been the focus of much research but is still not fully understood [157]. The most accepted hypothesis is interference with the detoxification of heme to hemozoine [153].

1.7.2.2 4-Aminoquinolines

Chloroquine is the main 4-aminoquinoline used clinically. At first it was thought to be too toxic for human use, but this was reconsidered during the Second World War. Until a decade ago, chloroquine was the first-line treatment in most parts of the world. Today, the extensive spread of parasite resistance has severely limited its use. Several hypotheses have been proposed to explain the mechanism of action [157]. As for the quinoline-methanols the most probable mechanism is interference with hemozoine formation, probably by heme/hematin-binding resulting in parasite death by heme/hematin poisoning [158, 159].
1.7.2.3 8-Aminoquinolines

Primaquine, derived from methylene blue [156], is so far the only drug on the market that can effect a radical cure by killing the hypnozoites. Alternative 8-aminoquinolines (e.g. tafenoquine) [161] are under clinical development. The mechanism of action is unknown but is proposed to involve an effect on parasite mitochondria [157, 160].

1.7.2.4 Phenanthrenes

This class was found to be active as antimalarials during the drug discovery efforts of the Second World War. However, due to the efficiency of chloroquine, halofantrine was not marketed until 1988. Adverse cardiac effects and high price have limited its use [158, 162]. Halofantrine has a blood schizonticidal effect, but the mechanism of action is still unknown [157, 161].

1.7.2.5 Artimisinins

These compounds are related to artesinin, a sesquiterpene derived from the herb Artemisia annua, which has been used historically in China as a treatment for malaria [163]. In addition to the natural artesinin, semisynthetic derivatives have been increasingly employed during the past 20 years [164]. The antimalarial action is mediated by free radicals and involves covalent linkage of artesinin to parasite membranes, proteins, and heme [157, 164, 165].
1.7.6 Antifolates

This class can be further divided into two separate groups depending on their activity on the parasite’s folate pathway: inhibitors of DHFR, e.g. pyrimethamine and proguanil, and inhibitors of DHPS including the sulphonamides, e.g. sulfadoxine, and the sulfones, e.g. dapsone [157]. A combination of these two groups, sulfadoxine-pyrimethamine (SP) is currently the first-line treatment in many parts of Africa [158].

1.7.7 Antibiotics/ Tetracyclines

With the increase in drug resistance, the use of some antibiotics has been reevaluated [157]. The most commonly used antibiotics are tetracycline and doxycycline. These are generally used in combination with other drugs [158].

1.7.3 Drug-resistant P. falciparum malaria

For several decades, the gold standard for the treatment of malaria was CQ, a 4-aminquinoline that was previously characterized by its efficacy, low toxicity and affordability (less than US $0.2 for a three-day adult treatment course) [166]. CQ acts by
binding to haem moieties produced from proteolytically processed haemoglobin inside infected erythrocytes, thereby interfering with haem detoxification \([167, 168]\). Massive worldwide use of CQ, beginning in the late 1940s, was followed a decade later by the first reports of CQ-resistant strains of \(P. falciparum\) \([169]\). Today, CQ resistance has spread to the vast majority of malaria endemic areas, rendering this drug increasingly ineffective. However, in spite of the prevalence of CQ-resistant \(P. falciparum\), this drug continues to be widely used. This is particularly problematic in sub-Saharan Africa, where resource limitations are profound and where highly immune populations often seem to respond at least partially to CQ therapy, and therefore somewhat mask the spread of resistance. CQ resistance almost certainly contributes to the recent finding that malaria-associated mortality is on the increase in Africa \([170]\). Sulphadoxine-pyrimethamine (SP), a combination Antifolate drug, is the only other widely used inexpensive antimalarial, but resistance is also leading to unacceptable levels of therapeutic failure in many areas in Asia, South America and now Africa \([171]\). Despite some optimism about new drug development for the future, as noted above, the malaria endemic regions of the world are faced with an unprecedented situation in which the only affordable treatment options are rapidly losing therapeutic efficacy.

### 1.7.4 The urgent need for new antimalarial agents

New antimalarial drugs must meet the requirements of rapid efficacy, minimal toxicity and low cost. Immediate prospects for drugs to replace CQ and SP include amodiaquine (a CQ-like quinoline) and chlorproguanildapsone (LapDap, another antifolate combination that inhibits the same enzymes as SP). These replacements will probably provide a few years of efficacy, particularly in Africa, but they already suffer from some crossresistance with CQ and SP, which increases the likelihood that full-blown resistance to these drugs will emerge rapidly \([172-174]\). High on the list of mid-term replacements are artemisininn derivatives. However, these drugs have very short half-lives, which necessitate their use in combination with a longer acting drug. Clearly, additional new drugs are needed. If we are to avoid an ever-increasing toll of malaria on tropical areas, it is imperative to rapidly put into action strategic plans for the discovery and development
of novel antimalarial compounds that are not encumbered by pre-existing mechanisms of drug resistance.

1.7.5 The desired profile for new drugs

Ideally, new drugs for uncomplicated *P. falciparum* malaria should be efficacious against drug resistant strains, provide cure within a reasonable time (ideally three days or less) to ensure good compliance, be safe, be suitable for small children and pregnant women, have appropriate formulations for oral use and, above all, be affordable [175, 176]. Drug development necessarily requires trade-offs among desired drug features, but for the treatment of malaria in the developing world the provision of affordable, orally active treatments that are safe for children is, for practical purposes, mandatory. Cost drives the choice of drugs in most developing countries, especially Africa, where most people must survive on less than US $15 per month.

Additional desirable uses include Intermittent Preventive Treatment in pregnancy and childhood, treatment in refugee camps and other emergency situations, treatment of severe malaria, and the treatment of malaria caused by *P. vivax* (a rarely lethal, but nevertheless debilitating and widespread, agent of malaria). Of less importance to public health, but potentially offering profitability, new drugs should ideally also provide protection against malaria when used as Chemoprophylaxis by advantaged non-immune populations travelling to endemic areas.

1.7.6 Future perspectives

Efforts to discover and develop new antimalarial drugs have increased dramatically in recent years, both as a result of the recognition of the global importance of fighting malaria, and the dedicated public–private partnership strategy to discover, develop and deliver new drugs. Increased funding from the public sector and philanthropic agencies has fuelled strong academic engagement in drug discovery, and increased partnerships with pharmaceutical companies mean that sets of complementary expertise are becoming available to drive and sustain the development of new drugs for diseases of low commercial return. Yet at the same time malaria mortality is on the increase due in large
part to the increasing ineffectiveness of the two first-line drugs, CQ and SP, and the current lack of affordable alternatives. Current enthusiasm for combining scientific innovation with expertise in the drug discovery and development process offers hope that a concerted effort can allow us to gain the upper hand in treating this disease. Time is a cruel judge, and we cannot afford to miss the current window of opportunity to develop new, affordable and effective antimalarial drugs.
References


3814.
3814.
[64] J. Bernstein, W.A. Lott, B.A. Steinberg, H.L. Yale, Am. Rev. Tuberc. 65 (1952)
357.
Rev. Tuberc. 65 (1952) 511.
Dis. 83 (1961) 891.
[73] A.E. Belanger, G.S. Besra, M.E. Ford, K. Mikusova, J.T. Belisle, P.J. Brennan,
Fowst, Arzneimittelforschung. 21 (1971) 1907.
285.
[77] D. Libermann, M. Moyeux, N. Rist, F. Grumbach, Seances Acad. Sci. 242
(1956) 2409.
[78] A. Banerjee, E. Dubnau, A. Quemard, V. Balasubramanian, K.S. Um, T. Wilson,