Molecular Mechanism of Drug Resistance

Shilpa Ray, Susmita Das, and Mrutyunjay Suar

Abstract The treatment of microbial infections has suffered greatly in this present century of pathogen dominance. Inspite of extensive research efforts and scientific advancements, the worldwide emergence of microbial tolerance continues to plague survivability. The innate property of microbe to resist any antibiotic due to evolution is the virtue of intrinsic resistance. However, the classical genetic mutations and extrachromosomal segments causing gene exchange attribute to acquired tolerance development. Rampant use of antimicrobials causes certain selection pressure which increases the resistance frequency. Genomic duplication, enzymatic site modification, target alteration, modulation in membrane permeability, and the efflux pump mechanism are the major contributors of multidrug resistance (MDR), specifically antibiotic tolerance development. MDRs will lead to clinical failures for treatment and pose health crisis. The molecular mechanisms of antimicrobial resistance are diverse as well as complex and still are exploited for new discoveries in order to prevent the surfacing of "superbugs." Antimicrobial chemotherapy has diminished the threat of infectious diseases to some extent. To avoid the indiscriminate use of antibiotics, the new ones licensed for use have decreased with time. Additionally, in vitro assays and genomics for anti-infectives are novel approaches used in resolving the issues of microbial resistance. Proper use of drugs can keep it under check and minimize the risk of MDR spread.

1 Introduction

With the advent of technological advancements, the rising scientific era witnessed the emergence of infectious diseases. This led to a sharp increase in global mortality and morbidity rate. Hence the research community held up the war against these pathogens by investigating deep into their molecular mechanisms, their host–pathogen interaction, and their epidemiology for the discovery of fine effective antimicrobial measures for host survival and safety. The researchers treated the

S. Ray • S. Das • M. Suar (✉)
School of Biotechnology, KIIT University, Bhubaneswar, Odisha, India
e-mail: msuar@kiitbiotech.ac.in

G. Arora et al. (eds.), *Drug Resistance in Bacteria, Fungi, Malaria, and Cancer*,
DOI 10.1007/978-3-319-48683-3_3

pathogenic ailments with useful inventions for long-term medication. Drug generally implies to foreign elements or agents that have some medicinal properties for common therapeutic usage. They can be used for bacterial infections, even as antifungal or antiparasitic agents, for cancer treatments, etc. The discovery of antibiotics was the greatest medical intervention affecting human survivability and health regime (Chain 1979; Fleming 1944; Dougherty and Pucci 2011; Ligon 2004). However indiscriminate usage had dramatically introduced new biological problems that are hard to confront with the present-day scientific solutions. Hence failure of medications did set the dawn of a post-antimicrobial era. The time of the Second World War had limited access to these expensive, rare, systemic medications (sulfonamides, penicillin, etc.). With time, simplified production of formulations eased the use of such treatments. Gradually, these antimicrobial agents mostly antibiotics became the elixir for the ailments from time then (Dougherty and Pucci 2011). Moreover the discoverer of penicillin Sir Alexander Fleming warned the surfacing of resistant forms of *Staphylococcus aureus* due to improper penicillin usage which would cause serious host complications (Hartman and Tomasz 1984). Few years later resistant forms emerged with 50 % of susceptible strains becoming resistant to the drug. Similar trend was observed in many other microbial species switching their drug sensitivity approach to a severe resistance mechanism thereby affecting healthy non-vulnerable population. This section will discuss in detail the emergence of drug-resistant microbial populations and the factors that govern their drug-resistant feature. The major focus of this segment will highlight the molecular, cellular, clinical, and genetic factors that bring about this severe cause of drug resistance. Beginning from the natural microbial resistance to the evolutionary alteration in the pathogen's genome, this chapter will cover the idea of how dealing with the conventional drug resistance mechanisms in the twenty-first century will create new frontiers for innovative therapeutic development. The problems and the complex challenge of dealing the multidrug resistance (MDR) mechanism at the molecular level will enable strategies for futuristic drug development for combating fungal, bacterial, and viral resistance mechanism.

2 Emergence of Drug Resistance: The Road So Far

Adaption is a very essential condition for survival as well as sustenance. All living organisms nurture themselves with crucial components from their living system. In addition to fundamental requirements, adaption against the toxic agents also requires armors of endurance. The adage "survival of the fittest" also applies to the environmental sustenance of microbes. This microbial tolerance has enabled the mechanism of resistance as one of the means to combat the harmful environmental effects. This results in conferring multiple drug resistance within pathogens against idle treatments. The first drug resistance occurred against penicillin and sulfonamides against *S. aureus* (Rammelkamp and Maxon 1942; Sabath et al. 1977). The discovery of antibiotics led to the emergence of antibiotic resistance in the

following two or more decades. The pathogens in the hospitals were not only reported to be resistant to the therapeutics but also remained viable for further infecting the vulnerable individuals with weakened immune system. The nineteenth century had an impressive pattern of increased tolerance mechanism among the pathogens from sulfonamide and penicillin-resistant *S. aureus* to multidrug-resistant *M. tuberculosis*. Some gastroenteric pathogens like *Shigella*, *Salmonella*, *V. cholera*, *E. coli*, *P. aeruginosa*, etc., also developed resistance against many antimicrobials during the course of time. Some strains also enabled community-dependent infection spread like *Streptococcus* developing resistance to penicillin and *S. aureus* and *Enterococcus* developing resistance to vancomycin.

2.1 History of Antibiotic Resistance Development: A Problem Getting Worse

The emergence of drug resistance has always been a major concern worldwide right after the introduction of drugs for common use. The crucial role of microbes in causing diseases led to the discovery of antimicrobial drugs (Davies and Davies 2010). Penicillin was the first of its kind as mentioned before to be introduced by Alexander Fleming in 1928 (Fleming 1944). Its effectiveness was against the Gram-positive bacteria, especially *Staphylococcus aureus* followed by few more antibiotics including streptomycin, tetracycline, chloramphenicol, vancomycin, macrolides, nalidixic acid, etc. (J. T. Park and Stromistger 1957). However, different drug-resistant microorganisms also started to show up with due time course. In the 1950s, penicillinase-producing *S. aureus* found its way to the society resulting in the gradual emergence and spread of multidrug-resistant *S. aureus*. To combat the harmful effects of penicillinase-producing *S. aureus*, methicillin was developed, but to utter disappointment, methicillin-resistant *Staphylococcus aureus* (MRSA) thwarted mankind in the UK (Brumfitt and Hamilton-Miller 1989; Klevens et al. 2007). Meanwhile, ampicillin and piperacillin were produced as broad-spectrum antibiotics, which also proved to be effective against Gram-negative *Enterobacteriaceae* and *Pseudomonas aeruginosa*, respectively. In the 1960s, a new genre of drugs named cephems was designed and widely used (Bryskier 2000). With time, different generations of cephems were developed according to their antimicrobial spectra. But simultaneously, there was emergence of penicillin-intermediate *S. pneumoniae* (PISP) in the latter half of 1967 and penicillin-resistant *S. pneumoniae* (PRSP) in the 1970s. Frequent use of cephems was believed to be responsible for the increase of PRSP. Ampicillin, which was earlier effective against *Haemophilus influenzae*, failed in the 1980s, when the strains gained resistance against antibiotic by producing β-lactamase (Rubin et al. 1981). In the 1990s, β-lactamase-producing strains reduced in Japan, but highly resistant β-lactam strains increased in number through mutations in their PBP (penicillin-binding protein) genes. Such were named β-lactam-negative ampicillin-resistant

(BLNAR) strains which were more common in Japan than in other parts of the world. In the latter half of the 1990s, vancomycin-resistant *S. aureus* (VRSA) was reported in the USA, which was thought to acquire the antibiotic resistance gene from vancomycin-resistant enterococci (VRE) via horizontal gene transfer (Goldrick 2002; Sung and Lindsay 2007). In the early twenty-first century, the increased use of third-generation cephems and carbapenem, quinolones gave rise to increased risk of resistant *Gonococci*, multidrug-resistant *P. aeruginosa* (MDRP), and quinolone-resistant *E. coli*. A relatively new concern in this field is the multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis. According to 2013 reports, out of all TB cases worldwide, approximately 5 % were estimated to be MDR-TB where the bacterium is resistant to minimum two of the most powerful first-line anti-TB drugs, isoniazid and rifampicin (Klopper et al. 2013). When MDR-TB becomes resistant to at least one drug from each group of second-line anti-TB drugs like fluoroquinolones and other injectable drugs, it's defined as XDR-TB. Apart from resistance in microbes, resistance to chemotherapy by cancer cells emerged to be a major concern in cancer research.

3 Factors for Drug Resistance Development: The Ongoing Phenomena

Antibiotic resistance is a serious global issue that has seized the roots of development. Antimicrobial resistance affects host immune profile, modulates with pathogen's fitness cost, and influences the genetic co-selection of resistant species with their frequency of reversibility potential (Andersson and Hughes 2010). The biologic mechanisms of the microbe are mostly responsible for such a resistant feature to fight the environmental toxic conditions. The inherent property of the pathogen, i.e., the natural resistance of the microbe, is a reason of resistance emergence. The major causative factor of resistance development is also the frequency of appearance of resistant bacteria due to genetic mutations or evolutionary horizontal gene transfer (Dzidic and Bedeković 2003; Thomas and Nielsen 2005). These mutations can modulate with the pathogen's drug uptake and efflux ability along with target alteration. Secondly the exposure of the pathogen to the drug/therapeutic influences the screening of resistant strains. The drug pharmacokinetic properties which affect pathogen's sustenance and clearance directly measure the degree of resistance mechanism. Human microflora is a hub of microbes and the release or exposure of wide-spectrum antibiotics can trigger resistant microbes to flourish and spread their tentacles of tolerance. Antibacterial agents mostly target bacterial cell wall synthesis, protein synthetic machinery, DNA duplication, and repair processes or transcriptional regulatory processes. Thereby the resistant mutants lack biological fitness. Hence, the natural selection theory suggests less prevalence of antibiotics to decrease the emergence of resistant species. Even the frequency with which the genetic alterations occur within the microbe affecting varying degree of

resistance is another factor. The antibiotic *selection pressure* is another factor of modifying the host's microbiota. The host system is subjected to a phenomenon of "selective pressure" when treated with antibiotics during infection. With a greater activity scale, the resistance frequency increases. This results in the resistant species surviving in the host population as compared to the susceptible strain with the harsh effect of the antibiotic. So being a reservoir increases the chances of infection spread to a greater extent. Antibiotics like cephalosporins, azithromycin, and fluoroquinolones bring about this effect of "selective pressure" in eukaryotic hosts. A careful and considerate use of antimicrobials is highly recommended for human safety. This high-end technical and materialistic world should concern the appropriate prescription of a much selective and narrow-range antibiotic thereby minimizing the risk of resistance development. Other factors include the drug exposure properties and concentration dosage dealing with their pharmacokinetic profile, the drug-pattern usage and its distribution scale, the immune system of the host (immunocompromised individuals are more prone to infection), pathogen's fitness cost of tolerance, and influence of nonresistant therapeutics, thereby affecting the pathogen's transmission intensity. However the poor and developing nations are reeling under the burden of drug resistance which directly affects their economic turnover. As a consequence, one has to raise the standards of high-end sophisticated health services and prevent the prevalence of community-associated infections.

Different factors acts as fulcrum in the process of drug resistance development (Wright and Poinar 2012). But which factors determine the most influential parameter for developing resistance and which factors remain insignificant are still under investigation. It's yet to be defined that how adaptation enable the microbe to culminate their survival strategy with resistance development. The understanding of the molecular and clinical mechanisms that ignite the "trigger responses" for microbial acclimatization has just begun. The literature of therapeutic invention has led to the conclusion that the once susceptible strains have developed weapons of resistance against the sensitive drug. For instance, a span of five decades made *Streptococcus* species resistant to penicillin and *S. aureus* resistant to vancomycin. This will enable one to formulate new mechanisms for restructuring new designs for drug development against many life-threatening diseases. Among the risk factors responsible for the emergence of drug resistance, illogical and rampant use of antibiotics is one of the crucial reasons for such an issue (Alanis 2005). The uncontrolled application of drugs in agricultural industry and in animals is becoming another rising factor for not only food production but also resistance development. Moreover treating immunocompromised individuals with life-saving drugs, increasing the survival chances of patients with unrestrained drug usage, and greater usage of invasive processes are other factors adding to the therapeutic tolerances. Greater medical advancements have led to control of infection and increased life expectancy of many patients. Even minor causes like lack of effective methods of hygiene and restrictions while handling infected patients can minimize the risk level of resistance to some extent. Not only widespread use of antibiotics but also harsh chemicals like herbicides, organic pesticides, and other toxic agents

along with chemotherapies for cancer and viral, parasitic, and fungal treatments have raised the concern for resistance emergence. The resistance mechanism in microbes, plants, and humans portrays certain homology in the proteins conferring such tolerance mechanism. Microbial genetics play a central role in shaping up the molecular structure for centralizing the mechanistic studies of drug resistance. The horizontal gene transfer influencing resistance linkage enables the co-selection of resistant species with tolerance to more than drug (Alonso et al. 2001; Baker-Austin et al. 2006). Reduced use of one drug can't revert back the resistance if the genetic alterations already support other resistance genes. Moreover co-selection can also lead to clonal substitution of the resistance-linked allele. Similar structural framework of drugs can even confer co-selection. This again challenges the regulatory checks. Drug resistance can be categorized into intrinsic and acquired resistance. The detailed mechanistic approach will be discussed in the sections ahead. Intrinsic mechanism deals with the natural ability of the microbe as an innate immunity mechanism, whereas acquired mechanism deals with the environmental influence bringing about genetic modification, thereby giving a new dimension to resistance aspect.

4 General Mechanism of Drug Resistance

Drug development still forms the top headed research enterprise globally due to unsuccessful therapeutic reign of potent drugs over microbial weapons. The term "drug" is generally applied to all foreign chemicals including antibiotics, herbicides, and therapeutic agents against virus, parasites, cancer, etc. The host–microbe warfare has led to the compromise of clinical interventions and rise of multidrug-resistant species (*Streptomyces*). Resistance to seven or more antibiotics has even led to a resistance phenotype for around 20 drugs. Such mechanisms have made the environment emerge into a reservoir of pathogen tolerance. The emergence of new infectious agents causing AIDS, SARS, etc., has modulated the resistance standards with raised clinical challenges. The fast-growing drug resistance mechanism will become the signature of potent microbes inhabiting the environment with new emerging diseases and higher tolerance level causing mortality and morbidity. Understanding of microbial genetics and gene manipulation modes will give a greater insight and provide a new dimension into fighting the resistance mechanisms (Hayes and Wolf 1990). The molecular mechanism of resistance can be categorized into *intrinsic* and *acquired* mode of tolerance. Intrinsic relates to the inherent and integral property of the microbe that has built up evolutionarily for resistance characteristics (Cox and Wright 2013). Additionally the procedure of mutations and selective characterization forms the major genetic changes for resistance emergence. Methods of gene transfer, gene alterations in stress-regulating genes causing altered protein expression, and gene amplification can bring about the change in the pathogen's genetic constitution. Both the molecular

approaches of resistance mechanisms will be discussed in detail in the following sections.

4.1 Intrinsic Resistance

Intrinsic resistance is defined as the ability of an organism to resist the antimicrobial/chemical compounds using a characteristic feature, which is an inherent or integral property developed by virtue of evolution. This can also be referred to as "insensitivity" due to the invulnerable nature of the organism toward that particular drug. The natural resistance feature, though less prevalent, sometimes undergoes spontaneous genomic alterations due to the absence of antibiotic-based selective pressure. However mostly the antibacterial-based microecological pressure triggers the stimulus for pathogen adaptation by the development of drug resistance. Mutations or evolutionary competition enables drug resistance gene uptake. It can arise due to certain events as outlined in Fig. 1 and mentioned below:

Absence/Modification of Target Site Microbial uptake of an antimicrobial drug is essential for a target-oriented action. Porins serve as the passageways for the drugs to cross the outer membrane of the bacterial cell. Some bacteria have the ability to manipulate their cell wall or membrane in order to protect themselves

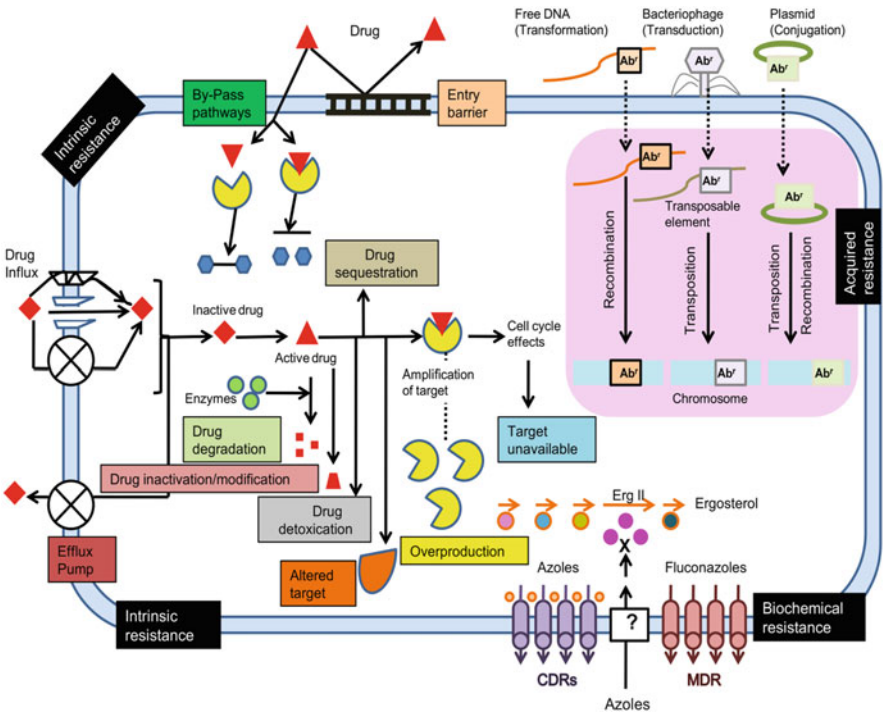

Fig. 1 Schematic presentation of multiple diverse molecular mechanism of microbial resistance

from foreign drugs. For example, certain Gram-negative bacteria can significantly lessen the uptake of certain antibiotics like aminoglycosides by altering the membrane porin frequency, size, and selectivity. On the other hand, the modification in the PBP (penicillin-binding protein) site led to the insensitivity toward the β-lactam antibiotics (Malouin and Bryan 1986).

Species-Specific Structure of Target Site Although the mode of action of antibiotics is almost similar across the same community of bacteria, species specificity has been detected in some cases. This is due to the lack of affinity of the drug to its target site. Different species under a single genus of a bacterium can alter the binding site of the drug by presenting various structural motifs for the same target, thus developing resistance. For example, the crystal structures of the large ribosomal subunit in *Staphylococcus aureus* showed specific structural motifs and binding modes for different antibiotics of same function as well as for a particular drug against different species of the bacteria.

Inactivation of Antimicrobial Agents via Modification/Degradation Destroying or manipulating the active component of the antimicrobial drug has always been considered as one of the effective techniques adopted by microbes for protection. For example, in penicillins and cephalosporins, the bacterial enzyme beta-lactamase hydrolyzes and deactivates the beta-lactam ring producing inactive penicilloic acid. It is then unable to bind to the PBPs, thereby maintaining the cell wall synthesis of the bacteria (Waxman and Strominger 1983). This kind of inactivation has been observed in many Gram-negative and Gram-positive bacteria against chloramphenicol, aminoglycosides, etc., via acetylation, phosphorylation, and adenylation.

Presence of Efflux Pumps A drug needs to be inside a bacterial system in high concentrations for a longer period in order to exert a persistent effect. However, some bacteria take advantage of their highly efficient drug efflux pumps that act as an export or kick the drug out of the cell as soon as it enters leaving only a little trace of the drug, insufficient for any significant effect. Some pumps specifically extrude particular antibiotics such as macrolides, lincosamides, streptogramins, and tetracyclines, whereas multiple drug resistance pumps throw away a variety of structurally and functionally different drugs (Lewis 1994). Most drug efflux proteins belong to five distinct protein families: the resistance–nodulation–cell division (RND), major facilitator (MF), staphylococcal/small multidrug resistance (SMR), ATP-binding cassette (ABC), and multidrug and toxic compound extrusion (MATE) families (Stavri et al. 2007). Except for ABC transporters, efflux by proteins of the above mentioned families is driven by proton (and sodium) motive force and is known as secondary transport. On the other hand, the primary ABC transporters drive efflux through ATP hydrolysis. These strategies have been observed in:

(a) *E. coli* and other *Enterobacteriaceae* against tetracyclines
(b) *Enterobacteriaceae* against chloramphenicol
(c) *Staphylococci* against macrolides and streptogramins
(d) *Staphylococcus aureus* and *Streptococcus pneumonia* against fluoroquinolones

High Detoxication Capacity Many bacteria secrete toxic compounds to protect themselves from their predators and other competitors. But they also need to evade the harmful effects of those noxious chemicals they produce. This is seen in the case of antibiotic-producing bacteria such as *Streptomyces* spp. In their defense, they develop resistance involving inactivation of their own antibiotic products streptomycin and neomycin by phosphotransferases and acetyltransferases and also by protecting the target site, i.e., rRNA by methylation in erythromycin-producing *S. erythraeus*. Additionally, in higher organisms, protein expression related to protection against chemicals is highly tissue specific. For example, the mammalian lung withstands the damage due to oxygen-induced free radicals. So this tissue has developed a large number of defense mechanisms including glucose-6-phosphate dehydrogenase, α-tocopherol, glutathione, glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase, etc. Also, the bronchiolar epithelium contains high levels of detoxication enzymes due to its direct exposure to the environment. These features make sure that it has a natural resistance to many drugs that work through the generation of free radicals or perform as alkylating agents.

Low Drug Delivery This is due to low bioavailability and stability, fast metabolism, and less time for circulation of the drug inside the host system. All these things contribute to low drug delivery into the target site, but since the drug is exposed to the environment, resistance can be developed by the microbes or harmful cells such as tumor cells.

Cell Cycle Effects In mammalian cells, the rate of cell division is a major cause of intrinsic drug resistance. This is due to the fact that the main dose-limiting determinant in the cancer chemotherapy is the toxicity to the rapidly diving normal cells. Mostly, the anticancer drugs are effective against the quickly proliferating malignant cells. So, solid tumors that are slow growing develop resistance as most of the cells are in G_0 resting state.

Chemically Induced Adaptive Change Drugs or other types of toxic agents, upon entry into the cell, evoke many biochemical changes inside that lead to adaptation of the cells against the same or other compounds. The difference between intrinsic resistance by adaptive changes and other forms of intrinsic resistance is that the former is temporary and reversible in the absence of the toxic agent. This fact is observed in clinical practices, especially in cancer therapy where it protects the normal host cells but not the tumor cells from the adverse effects of the chemotherapeutic agent.

Stress Response Environmental factors other than drugs, such as pH, osmotic shock, UV irradiation, heat, trauma, viral infection, anoxia, and oxidative stress, can contribute to stimulate a genetic reflex in the cells that provide resistance not only to the stress factors involved but also against drugs. Prokaryotes have mainly four stress-induced regulons, namely, the SOS response, the oxyR network, the heat-shock response, and the adaptive response to alkylating agents. Like in *E. coli*, the *gro*EL and *dna*K heat-shock proteins are not only induced by hyperthermia but

also by UV irradiation or nalidixic acid. Both of them affect the SOS response. Similarly, in *Salmonella* Typhimurium, the ability of a cell to adapt to H_2O_2-induced oxidative stress also gives resistance to heat killing. Many biochemical events influence resistance development which mainly includes decreased drug delivery and uptake, high efflux as mentioned above, greater inhibition of metabolic drug activity, and drug sequestration mechanisms.

4.2 Acquired Resistance

Microbial drug resistance development is related to the organization of their genetic material that becomes tolerant and the ease of uptake of exogenous DNA to alter their inherent genetic makeup. The continued selective pressure has thereby led to different modes of pathogen survival against the harsh medications. The emergence of resistance mostly involves two categories of pathogen: one involving the susceptible group and the other heterogeneous group comprising at least one microbe with drug-resistant determinant. The resistant group emerges fit with renewed genetic composition coding for the resistance which further assists in its propagation. Efflux mechanisms, drug modulation, membrane permeability alteration, etc., form the bullets of superbug evolution as depicted in Fig. 1. Thus the MDRs like *Pseudomonas*, *Klebsiella*, methicillin-resistant *Staphylococcus aureus* (MRSA), and XDRs like *Mycobacterium tuberculosis* have evaded the clinician's arsenal with their remarkable virulence potential. For an insight into drug invention, an understanding of molecular mechanism of drug resistance will help to sort out the therapeutic trade-offs with novel approaches. As mentioned before, pathogen drug resistance mechanism can be either *intrinsic* or *acquired* (Fig. 1). The "biological" aspect of resistance development is either absent from a majority of microbial population or is underexpressed before drug exposure. Microbial resistance has its basis at the genetic level which is modified either by gene knockout or introduction. This alters the genetic composition and cellular gene expression forming myriads of biological resistance forms (Mazodier and Davies 1991). Where intrinsic mechanism is solely due to the inherent microbial property of natural chromosomal genes and efflux system, acquired mechanism involves genetic mutations or gene transfer/exchange methods through the process of *transformation*, *transduction*, or *conjugation* (Fig. 1) (Flintoff 1989). Conjugation, the most general mode of drug resistance transmission, is facilitated by plasmids by forming a temporary "pilus" between two adjacent bacteria for genetic material exchange. Transformation is the process of uptake of exogenous DNA from the surrounding due to microbial degradation/lysis for further incorporation into any recipient organism's genetic cassette. Transduction essentially requires a vector specifically viruses that carry up the drug resistance genes for further introduction into bacterial host (bacteriophage mode of resistance transmission). Gene transfer is not genus specific, so the divergence of genetic exchange has led to the evolutionary buildup of the resistance reservoir. The independently replicating plasmids distinguishable

with their origin of replication mostly contain the genes of antibiotic resistance. Transposons encoding the resistance determinants are the "jumping genes" present either on plasmids or host chromosome (Frost et al. 2005). The DNA terminal sequences enable recombination and encode proteins which facilitate their stable integration into host genome. Conjugative transposons bear unique plasmid-like properties which add on the advantage of funneling up many endogenous extra-chromosomal elements. Integrons comprise of gene cassettes that bear stable recombination features of undergoing multiple gene exchange within a single crossover. One super-integron was reported in *Vibrio cholerae* which comprised of about 3 % of host's genetic makeup. Numerous plasmids existing within a single microbe frame the genetic composition of the organism. They even comprise of "R factors" annotated as the resistance units forming the means of resistance spread among microbes. *Shigella* strains bearing self-replicating as well as self-transferable elements were identified to exhibit sulfonamide tolerance. Streptomycin, chloramphenicol, and tetracycline were used as optional medications; however the susceptible strains started developing tolerance against all three antibiotics with due time course.

After resistance gene transfer, the gene overexpression or mechanistic biological activity modulates the drug treatment in a way to neutralize its effect. The biological mechanism of resistance generally involves chemical/enzymatic degradation or modification of the therapeutic agent rendering it inactive against the bug. Such is the mode of resistance development in case of β-lactam antibiotics. Secondly, the active drug efflux mechanisms, much intensified than influx modes, promote effective resistance development. Efflux mode of microbial tolerance was evident in tetracycline and fluoroquinolones. Thirdly, target modification involves the microbe to alter the substrate binding affinity of the drug thereby hampering its activity. Similar mechanistic methods involve structural conformational changes in PBPs which renders penicillin resistance and DNA gyrase modulation which leads to fluoroquinolone tolerance (Wolfson and Hooper 1985). The frequency of mutations within the wild-type microbial population that has emerged irrespective of any selective pressure or drug exposure is attributed to the natural selection of emergence of acquired resistance. This differentiates from the intrinsic mechanism where the genetic alteration becomes a part of the biological variation. Selection of the microbial units that can withstand and sustain the chemical insult proves superior. Examples include the PBPs in *E. coli* causing cephalosporin resistance and alterations in acetylcholinesterase conferring tolerance to Rabon (Tripathi and O'Brien 1973). Drugs or antimicrobials aren't mutagenic. However, certain tumor treatments involve mutagens in chemotherapy that can evolve the selection pressure for resistance thereby causing genetic instability with high mutations or amplifications. In tumors specifically the differentiation between natural selection and acquired tolerance is a highly debatable topic. Such phenomenon leads to the constitutive expression of certain phenotypic changes instrumental for adaptive response. In *E. coli*, the altered LexA repressor gene has an impact in regulation of SOS signals (Little and Mount 1982). Even *S.* Typhimurium when resistant to hydrogen peroxide modulates the expression of certain stress-regulating genes

including catalase, SOD, glutathione peroxidase, etc. Changes at the transcriptional level or early mutational occurrence influence significant mechanistic cascades that lead to overexpression of proteins modulating the microbial genotype to display resistance phenotype. The modes of development of acquired resistance involve mutations, efflux systems, gene amplifications, drug modification, or target alterations.

Chromosomal-Based Genetic Alteration Mutation in drug targets is basically the most common mechanism of microbial resistance emergence. The fluoroquinolone resistance mechanism can be attributed to genetic alterations as well as efflux pump machinery. The drug targets DNA gyrase as well as topoisomerase IV which when altered confer fluoroquinolone resistance. These multi-subunit targets play a pivotal role during DNA duplication each comprising of two subunits: GyrA and GyrB for DNA gyrase and ParC/GrlA and ParE/GrlB for topoisomerase IV. One subunit of these complexes functions for the DNA-binding role, whereas the other carries up the ATP-binding and hydrolysis role. The quinolone-resistance determining-region in DNA-biding domain bears the mutational changes that confer antibiotic resistance. Innumerable mutations impose additive effects to build up the bacterium's resistant trait. Similarly rifamycins form the front-line therapeutic against *tuberculosis* infection either individually or in combination with isoniazid, streptomycin, etc. However, RpoB point mutations prevent the drug-binding affinity at the RNA polymerase subunit conferring combinatorial drug resistance (Mariam et al. 2004). Sulfonamide targets dihydropteroate synthase whose alteration results in decreased enzymatic activity for the drug. Trimethoprim blocks dihydrofolate reductase enzyme whose mutation causes protein over-induction with reduced drug affinity. Point mutations at 16S rRNA and 23S rRNA operons confer tetracycline and MLS antibiotic resistance, respectively (Ross et al. 1998).

Genomic Duplication Gene alterations mainly include gene mutational events along with gene amplification or overexpression. The method of genomic doubling is quite prevalent in conferring drug resistance among eukaryotic cells, the sole reason in tumors. This genetic induction leads to the modulation at the protein level for augmented biosynthetic machinery leading to the overexpression of many transporters. In *E. coli*, the genomic amplification of *acrAB* locus in tetracycline exposure led to induction of the AcrAB efflux pump systems contributing to multiple drug-resistant phenotype (Nikaido and Zgurskaya 2001). Such duplication phenomenon has also been observed in *S. aureus* with respect to methicillin resistance. Genome amplification forms one of the mechanisms of resistance avoiding the boundaries of mutational aspects. However the absence of drug made the microbes revert back to their normal phenotypes. So the tolerance mechanism is basically unstable.

Enzymatic Approach of Drug Modification General mechanism of drug modification involves two specific classes of enzymes; one group which causes drug degradation and another catalyzes chemical modifications. The β-lactamases encoded by plasmids and transposons confer adaptive resistance as compared to

the chromosomal chunk which attributes intrinsic property. The structural and functional characterization classifies β-lactamases into two groups, one having serine at the active catalytic site (classes A, C, D) and another with zinc-dependent metalloenzyme (class B). Being zinc dependent, class B enzymes are susceptible to EDTA and hydrolyze carbapenems. AmpC, a prototypic class C-type enzyme being plasmid borne, is easily transferred among many Gram-negative strains like *Salmonella* spp., *Klebsiella* spp., etc. (Jacoby 2009). Even the acetyltransferases, phosphotransferases, and adenylates that modify the aminoglycosides exist on certain mobile genetic units or integrons where they enable resistance transmission. The MLS antibiotics are inhibited by groups of esterases as well as phosphotransferases that modulate 14- and 15-membered macrolides (Nakajima 1999). The acetyltransferases and hydrolases affect streptogramin A and B drugs, respectively. The transferase enzyme concerned with nucleotidyl moiety transfer bestows resistance to lincosamides antibiotics. Even chloramphenicol, which targets the protein biosynthetic machinery, is a bacteriostatic drug which is inactivated by certain groups of acetyltransferases. Prevalent mostly in *Enterococcus* and *Staphylococcus*, the enzyme's translational attenuation depends on the regulation of their protein expression and induction.

Modulated Drug Targets β-Lactamase-producing *S. aureus* was the first penicillin- and methicillin-resistant strain. The mechanism involved genes contributing to changes in PBPs that confers β-lactam resistance in *Streptococcus* as well as *Staphylococcus*. This gene is encoded by *mecA* on a mobile genetic unit in resistant *Staphylococcus aureus* (Wielders et al. 2001). The "staphylococcal cassette chromosome" is the mobile element comprising of regulatory segments and enzymes responsible for site-specific recombination. The cell wall synthetic process requires a number of PBPs in *Staphylococcus aureus*. PBP2 enzyme plays dual role in resistant *S. aureus* where the transpeptidase and transglycosylase activities switch in accordance to drug exposure for imparting susceptibility or tolerance features in the microbe (Brown and Reynolds 1980). The plasmid-borne *qnr* determinants found widely in Gram-negative species of *E. coli*, *Shigella*, non-typhoidal *Salmonella*, etc., affect fluoroquinolone sensitivity (Piekarska et al. 2011). The pentapeptide repeat protein family includes Qnr as well as MfpA which regulate fluoroquinolone resistance by shielding DNA gyrase and topoisomerase II, respectively. Qnr additionally protects topoisomerase from drug effect. Moreover MfpA in *Mycobacterium* forms an identical structural outlook of B-DNA-inhibiting ciprofloxacin activity by interaction with DNA gyrase (Montero et al. 2001). Coupled with other modes, Qnr and MfpA can amplify the resistance profile to higher extents. Glycopeptides form the major example of bearing the drug resistance feature due to drug target modifications in Gram-positive spherical bacteria. Multiple clustered proteins contribute to resistance by modulating the peptidoglycan production. The complex elucidation of unveiling the glycopeptide resistance concerns gene clusters like racemases or dehydrogenases forming serine and lactate, respectively, which alter the peptidoglycan framework. The two-component unit regulates the cellular biosynthetic mechanisms. With intact

D-Ala available, the reduced interaction efficiency with serine and lactate substrates nullifies. The dipeptidase and carboxypeptidase act in accordance to their respective function of cleaving and removing the terminal D-alanine. The vanA gene cluster conferring vancomycin resistance in *Enterococcus* has transferred its resistance determinants even in *S. aureus* encoded on Tn*1546* transposon. However, plasmid from *E. faecalis* forms the initial mode of *vanA* transfer into *Staphylococcus*. The erythromycin resistance methylase (*erm*) influences the macrolide drug-binding affinity by methylation of adenine residues of 23S rRNA (Maravic 2004); Vester and Douthwaite 2001. The resistance markers are usually constitutively expressed or in certain cases MLS drug exposure induces expression.

Efflux Mechanisms and Membrane Permeability Channel Rather than restricting drug uptake and internalization, resistance is mostly due to the failure of undergoing drug interaction with cellular targets due to drug effusion (Fig. 1). Efflux pump mechanism ejects the drug out of the cell and was initially observed during tetracycline resistance development. The five protein transporter families involved in efflux machinery are ATP-binding cassette (ABC) transporters, resistance–nodulation–cell division (RND) protein superfamily, major facilitator (MF) protein groups, small multidrug resistance (SMR) units, and multidrug and toxic compound extrusion (MATE) protein superfamily (Poole 2005). ABC group of primary transporters employ ATP hydrolysis for efflux mechanism, whereas the rest secondary groups involve proton-motive gradient force for conferring drug expulsion (Kobayashi et al. 2001). The drug discharge proteins are either categorized into single protein component systems having narrow substrate range or bear two proteins to facilitate the binding of variable structural compounds conferring wide spectrum of resistance phenotypes. RND transporters enable efflux of cytosolic proteins through the inner and outer membrane barriers. The 20 tetracycline efflux transporters comprise of transmembrane spanning regions where the protein expression is regulated under the transcriptional repressor. The inactivation of the repressor by the drug promotes the expression of the tetracycline efflux machinery. TetA-E are the efflux proteins in Gram-negative bacteria, with TetK and L in Gram-positive microbes, and TetR is the repressor (Schnappinger and Hillen 1996). Certain proteins are also involved in electroneutral chemical reactions. Additionally, macrolide and streptogramin tolerance is attributed by a group of ABC efflux protein family called MsrA. Its homologues VgaA and VgaB in *Staphylococcus* bring about streptogramin A and pristinamycin resistance, respectively (Lina et al. 1999). Mef efflux systems also confer macrolide resistance in *Streptococcus*. Removal of efflux machinery can revert back the genetic profile to antibiotic susceptibility even with the persistence of chromosomal alterations. E1–E8 are the groups of efflux protein superfamily that confer phenicol resistance. CmlA is an efflux system aiding in chloramphenicol resistance, which promotes an induced attenuation-based resistance mechanism (Bischoff et al. 2005). Porin proteins enable the smooth flow of molecules across the cell membrane barrier in Gram-negative bacteria (Delcour 2009). OmpF in *Escherichia coli* and OprD in *Pseudomonas* act as checkpoints to monitor the nonspecific entry of many

compounds. OprD mutational changes lead to imipenem tolerance. Simultaneous expression and regulation of OprD and MexEF-OprM efflux complex will confer carbapenem resistance (Lee and Ko 2012; Quale et al. 2006). The bacterial cell envelop restricts and permits selective entry of many hydrophobic and hydrophilic components. Resistance to polymyxin B however doesn't involve porins but cell envelope alterations. The PmrAB system regulates the LPS and lipid A moiety changes which confers polymyxin B resistance in *Salmonella* Typhimurium (Gunn 2008).

5 Drug Resistance Mechanism (Disease Specific)

5.1 Tumor Drug Resistance: An Evolving Paradigm

After all the advancements in cancer research, emergence of drug resistance restricted the efficacy of the therapies. Resistance to cancer chemotherapy results from a range of factors, starting from individual variations in patients and somatic cell genetic differences in tumor, even those from the same tissue of origin (Dean et al. 2005). Resistance can be intrinsic as well as acquired. Chemotherapy resistance occurs when cancers that have been responding to a drug suddenly stop reacting. There are several possible reasons responsible which mainly include:

1. Some cancer cells that escape the harmful effects of the drug mutate and develop resistance toward the drug. Later, upon multiplication, they become more resistant.
2. Gene amplification: A cancer cell has the ability to produce hundreds of copies of genes of a particular gene. This leads to the overexpression of the corresponding protein, which in turn makes the anticancer drug ineffective.
3. With the help of a molecule called p-glycoprotein (P-gp), cancer cells pump out the drug entering the system using their proficient drug efflux pumps.
4. Highly efficient DNA damage repair machinery, one of the survival secrets of cancer cells, also plays vital role in contributing resistance against anticancer drugs (Holohan et al. 2013).
5. Cancer cells may also develop strategies to inactivate the drugs (Holohan et al. 2013).

The molecular mechanisms of drug resistance in tumors are discussed in details in the following segments:

1. *Altered membrane transport*: One of the most promising drug resistance mechanisms against antineoplastic agents is the method by which the cell flushes out the cytotoxic compound with the help of some membrane proteins that helps to reduce the inside drug concentration below the cell-killing threshold. These proteins modulate absorption, distribution, and excretion of many pharmacological compounds. ABC transporters are encoded by as many as 48 genes. In the

clinical transport-associated MDR, the most commonly involved gene is the MDR1 that encodes for the P-glycoprotein (P-gp; MDR1, ABCB1) which is a phosphorylated and glycosylated 170 kDa protein. Other well-known ABC transporters are the MDR-associated protein 1 (MRP1, ABCC1), the mitoxantrone resistance protein (MXR1/BCRP, ABCG2), and the ABCB4 (MDR3) and ABCB11 (sister P-gp or BSEP) proteins involved in the secretion of hepatic phosphatidylcholine and bile acids, respectively, as well as transport of certain drugs. The most interesting feature of differentiating MDR proteins from other mammalian transporters is their high substrate specificity. Unlike classical transporters, MDR transporters translocate a variety of structurally different hydrophobic compounds along with other unique compounds, and this forms the platform for the cross-resistance to many chemically unrelated compounds. Overexpression of MDR proteins in tumors like hepatomas and lung or colon carcinomas often shows intrinsic resistance (Gottesman 2002; Gottesman et al. 2002). The role of P-gp, in the absence of any therapeutic agent or toxin, is thought to protect the cell from xenobiotics. However, several reports suggest P-gp to have prognostic significance in certain types of neoplasms as well as to play an important role in CNS penetration of drugs (Begley 2004). But all these have failed to correlate with the clinical evidence. So, its mode of action has always been controversial. According to some reports, MDR proteins are not responsible for transporting drugs, but they alter ion transport or signal transduction, thus later on affecting drug distribution. In tumor cells, anticancer drugs and cytotoxic cytokines like TNF/Fas ligand family play an important role in induction of apoptosis and tumor therapy (Reed 2003). Drug-resistant tumor cell lines show resistance to Fas-induced caspase-3 activation and apoptosis which is reported to be mediated by P-gp. The cells expressing P-gp are resistant to a lot of stimuli responsible for the activation of caspase apoptotic cascade, whereas it is not the case in caspase-independent cell death where cell dies by the action of pore-forming proteins GzB.

2. *Genetic responses*: Drugs such as methotrexate inhibit key enzymes involved in the proliferation pathway of mammalian cells. When transcription of the gene encoding for the enzyme increases, large amount of enzyme is produced that leads to faster proliferation. But the concentration of the drug is limited which cannot block the additional enzyme that is produced. Thus the cells develop resistance against the drug. One way to overexpress the enzyme is by the amplification of the gene encoding the enzyme, which is achieved by replication of a region from the chromosome that results in multiple copies of the same gene. Several drug-resistant cancer cell lines and DNA from two drug-resistant leukemic patients have shown gene rearrangements in their chromosome resulting in the initial activation or enhanced expression of MDR1 gene. Moreover, therapy with rifampicin has also shown to induce MDR1 expression in healthy individuals. Hence, MDR1 overexpression can be affected by gene amplification/rearrangement, rifampicin induction, etc. Another important factor responsible in drug-resistant cancer is the mutation in the apoptotic gene p53. p53 usually induces apoptosis in cells which have undergone DNA damage

(Chen et al. 1996). Thus, the target DNA won't be affected and will continue replicating in the presence of mutated p53. So, the drugs that increase DNA damage will come to no use in certain cancers. In many cancer cases, deletion of p53 was reported to be linked to MDR. Also, reduced expression of p53 in human breast cancer cells altered response to paclitaxel and 5-FU. Other genes involved in apoptotic pathway, like h-ras and bcl2/bax, have also been observed to contribute to drug resistance (Davis et al. 2003). Thus drug resistance arising from genetic responses affects a large variety of anticancer drugs and increases the percentage of surviving mutant cells, which in turn leads to greater tumor heterogeneity.

3. *Enhanced DNA repair*: Cancer cells develop resistance to drugs such as cisplatin by an enhanced ability to remove cisplatin-DNA adducts and to repair the cisplatin-induced lesions with the help of certain DNA repair proteins like XPE-BF (xeroderma pigmentosum group E binding factor). ERCC1 (excision repair cross-complementing protein), a DNA-binding protein, has the ability to recognize cisplatin-induced DNA damage and thus its level increases in cisplatin-resistant cells (Siddik 2003). The level of ERCC1 is also found to increase in carboplatin-resistant tumors.
4. *Alterations in target molecules*: Modifications in the target of a drug is a common way to develop resistance against the same. As seen in antiestrogen (e.g., tamoxifen) therapy for breast cancer, patients undergo a transition from a responsive state to an endocrine-resistant state due to an apparent loss of estrogen receptors in the resistant cancer cells (Ring and Dowsett 2004). So they finally stop responding to tamoxifen treatment, while the growth of their tumors can still be inhibited for a short span by estrogen synthesis inhibitors like aromatase inhibitors followed by complete unresponsiveness to any endocrine modification. Hence, the surviving cancer cells no longer depend on estrogen for growth and the original drug that targets estrogen receptors becomes useless. Another example is a tyrosine kinase inhibitor, imatinib, which induces apoptosis in cancer cells by disabling the damaged bcr-abl receptors, preventing ATP binding (Capdeville et al. 2002). But reports suggest that during clinical trials, the chronic myeloid leukemic patients in remission had reactivated bcr-abl activity, few patients had amplified copies of the bcr-abl gene, and others had a single point mutation within the ATP-binding site of the gene. Hence, this gene shows to play an important role in initiation and maintenance of cancer and thus related to anticancer drug resistance (Dean et al. 2005). Mutation in the topoisomerase gene is another cause of drug resistance due to its vital role in DNA replication process. Chemotherapeutic drug like etoposide that targets topoisomerase II suffers from resistance when cancer cells mutate the latter in a way to alter its nuclear localization. Chromosomal losses are very common in cancer, and due to its aneuploid nature, there has been the emergence of MDR. While undergoing repeated cell division for a number of times, a cancer cell has the chance of losing the drug-sensitive gene from the chromosome, and also chromosomal rearrangement during mitosis can contribute to the activation or inactivation of different biochemical pathways that can affect the mode of action

of the drug. The size of the tumor also matters as the center part of most tumors has limited blood supply. So, the larger the tumor, the lower the drug efficacy. Apart from this, some metabolic enzymes, either alone or together with transporters like P-gps, can alter the drug absorption, distribution, metabolism, and excretion. For example, enzymes like cytochrome p450s (cyp450) in combination with P-gp greatly affect the drug absorption and bio-distribution to the tissues preventing intestinal transcellular permeability, biliary disposition in the liver, urinary elimination through the kidney, and placental transport.

5. *Metabolic effects*: Effective clearance of drugs can often be achieved by some xenobiotics that have the ability to modify high-density apolipoprotein or by overexpression of the drug-metabolizing enzymes and/or carrier molecules. The increased production of glutathione or ubiquitin leads to drug inactivity by the formation of conjugates that are excreted, for example, cisplatin that becomes resistant to ovarian carcinomas after the overexpression of dihydrodiol dehydrogenase. In some cases, the underexpression of few drug-metabolizing enzymes (e.g., deoxycytidine kinase) can also lessen drug (e.g., arabinosidase) activity in a situation where the drug needs to be catalytically cleaved to be in its active form. Additionally, protein kinase C has been found to have increased activity in the drug-resistant breast carcinoma cells because of its role in both drug exclusion and apoptosis (Caponigro et al. 1997). Breast cancer cells have shown resistance against paclitaxel and vincristine due to the involvement of the extracellular matrix as well. The ligation of the b1 integrins by the extracellular matrix inhibits apoptosis mediated by these two drugs.
6. *Growth factors*: High levels of serum interleukin-6 (IL-6) have been observed in different drug-resistant cancer cells, whereas cells sensitive to the chemotherapy did not produce any detectable IL-6. The reason behind this resistance was attributed to the activation of the CCAAT enhancer-binding protein family of transcription factors and induction of MDR1 gene expression (Okamura et al. 2004). Reports have also suggested that extracellular factors can contribute to drug resistance against a particular cancer. Like increased levels of acidic and basic fibroblast, growth factors in the media of solid and metastatic tumors can affect the broad-spectrum drug (paclitaxel, doxorubicin, and 5-FU) efficacy and lead to develop resistance. When applied in combination, these two growth factors can give rise to a tenfold increase in drug resistance.

5.2 *Antibiotic Resistance: The Bacterial Weapons*

The era of the twentieth century witnessed the discovery of essential antibacterial drugs to control bacterial proliferation for limiting infectious agents. Even though vaccines and other public health agendas were instrumental, still antimicrobial therapy checked the further transmission of infectious pathogens (Donadio et al. 2010). Antibiotics literally implies “against life” but scientifically are compounds that hinder with the normal functioning of the bacterium without interfering with

the biological processes of the eukaryotic host harboring the microbe (Fischbach and Walsh 2009). The present scientific world now struggles to combat the issue of antimicrobial resistance. The frequency in resistance has constrained the mob to question the efficacy of these conventional medications. The discovery of every drug is followed by the bacterial strategic mechanisms to overcome the stringency by developing tolerance. This has also led the researchers to investigate into the pathogen's clinical, molecular, and cellular factors that make them the superbugs of resistance (Arnold 2007; Neu 1992). Hence the new drugs have raised question on their proficiency to avoid the emergence of multidrug resistance microbes. The MDR pathogenic strains of *M. tuberculosis*, *S. pneumonia*, *S. aureus*, etc., have posed innumerable challenges for further antibiotic development (Wright and Poinar 2012). With few antimicrobials in hand, the post-antimicrobial era seems to be approaching soon. Antibiotics can be either bacteriocidal (bacterial death) or bacteriostatic (bacterial growth inhibition). The last five decades had enabled the discovery of many natural antibiotics like fungal penicillins and cephalosporins that kill the bacteria. Even streptomycin, tetracycline, etc., are known microbial targets from *Streptomyces*. Certain semisynthetic alterations led to the production of second- and third-generation antibiotics like β-lactams of penicillin and azithromycin from erythromycin. However, a complete synthetic antibiotic like ciprofloxacin came later into existence.

The targets of antibacterial drugs and their mechanism of action will help one to understand the emerging resistance profile among these bugs. The antibacterial drugs normally target the genes responsible for *bacterial cell wall synthesis* (Green 2002), genes involved in *protein biosynthetic pathway*, and the ones modulating the *microbial DNA replication and repair*. The rigid, flexible peptidoglycan lining of the bacterial cell is a meshwork of peptide and glycan cross-links which provides integrity and osmotic balance to the microbe. The transpeptidases and transglycosylases act on the amide and glycan links, respectively, to strengthen the osmotic rigidity of the cell. Both these enzymes are the antibacterial targets of β-lactam in penicillin and cephalosporin. These pseudosubstrates enable acetylation at the enzymatic active site which leads to weak cross-linkage of peptide bonds in the glycan lining, thereby making the cell susceptible to lysis. Even vancomycin targets the peptidoglycan layer, but not the cross-linking factor rather alters the substrate interaction with the enzyme. Target alteration weakens the cell integrity subjecting the bacteria to lysis. The high reactivity of vancomycin is due to the hydrogen bonds with the D-alanine dipeptide of peptidoglycan side chain. Both β-lactam and vancomycin work synergistically on the substrate and the enzyme when used in a combined recipe. Secondly, with distinct prokaryotic RNA and protein synthetic machinery, certain classes of antibiotics like erythromycin, tetracycline, and aminoglycosides selectively target the microbial survival by hindering with essential steps of ribosomal functioning. The new class of protein synthesis inhibitor have a huge spectrum of antibacterial target as the ribosomal machinery involves the protein synthesis steps of initiation, elongation, and termination of codons to build up the peptide chain. So the protein synthesis inhibitors target such large supramolecular machinery with essential biosynthetic process that further

alters the binding and catalysis of many enzyme-catalyzed reactions. The third group of antibacterial compounds disrupt with the DNA doubling and repair mechanism. For instance, DNA gyrase that helps in DNA strand uncoiling is targeted by the fluoroquinolones like ciprofloxacin. DNA topoisomerases are categorized as Type I and Type II in accordance to the single- or double-stranded breaks. Moreover, DNA gyrase is a Type II topoisomerases, and ciprofloxacin acts by forming a complex between the transient double-stranded break and inactive enzyme. Since the cleaved DNA mounts up, an SOS repair mechanism ultimately leads to the death of the microbe. Similarly topoisomerase IV is a major target in *Staphylococcus aureus*. The antibacterial drugs alter and modulate with the cell wall, protein, and DNA synthetic pathway. They act in a selective biochemical manner to target the microbial machinery as compared to that of host. The present generation of antibiotics not only require better efficacy and less toxicity use but also new unaltered targets for universal and regulatory acceptance.

The epidemiological studies have implicated the feature of selective advantage for pathogens. For instance, prior antibiotic exposure is a crucial factor causing salmonellosis. Secondly, antibiotic-resistant microbes are known to be more virulent with an overexpression of adhesins and toxins on R-plasmids thereby increasing the scale of virulence. Resistance can also lead to increased frequency of the disease. An infected individual with MDRs will be the carrier of infection causing transmission risk as compared to the susceptible strains. So reservoirs are quite important in the persistence of infectious agents. They also enable genetic elements swap and selective pressure exposure adds on to the evolution of resistance mechanism. A study hereby reported the clinical isolates of *S. aureus* and *S. epidermidis* to have similar drug-resistant profile obtained from the same hospital source during *S. aureus* epidemic (Cohen et al. 1982). Hence *S. epidermidis* served an important reservoir affecting the emergence and chances of resistance occurrence. Treating drug-resistant microbes requires more effort and expenditure for long-term antibacterial medications. Literature has suggested variant microbial properties, environmental factors, and frequent use of antimicrobial agents to be responsible for the incidence of drug-resistant microbes. A case occurred where a woman under the medication of chloramphenicol died due to chloramphenicol-resistant *S.* Typhimurium infection (Tacket et al. 1985). So chloramphenicol was no longer the drug option against salmonellosis. This further leads either to the emergence, persistence, or transmission of resistant ability. A case illustrates that the medications with cephalosporins lead to the augmentation of resistance in enterococci (Dahms et al. 1998). Where reservoirs are concerned, infants in nurseries serving as a depot for producing greater number of *staphylococci* were termed as “cloud babies.” Even certain microbes are posing health problems due to high-end technological and societal advancements which are influencing their resistant transmission. Economic changes are also a major factor destabilizing the health infrastructure in many advanced countries like the USA. These enable the application of certain control programs for the curtailment of pathogenic infections.

5.2.1 Survival Strategies

The invention of an antibiotic and its widespread acceptance for clinical use have limited time period for their medications. From months to few years, these antibiotics have proven to emerge resistant to their targeted bacterial strain (Bush et al. 2011). For instance, penicillin developed resistance among the bacterial populations within a span of 2 years after its global use. Similarly, vancomycin resistance against enterococci did spread at an alarming rate within 5 years of its introduction during the late 1980s. The resistance involves a collective action of five genes, the major reason why vancomycin resistance took a greater time to evolve (Walsh et al. 1996). The clinically important resistance is dependent bacterial doubling time, where the intrinsic resistance can lead to genetic alterations in 1:100 ratio finally amassing a pool of resistant superbugs. In this population, if the resistant species proves superior and tolerates the antibiotic, then the pool of sensitive population are killed where the resistant species fills up the numbers of the susceptible ones with their own dominant resistant type. A subtherapeutic medication assures practical resistant species outgrowth. Antibacterial resistance generally involves four basic mechanisms of target modification, drug inactivation, decreased drug uptake, and increased efflux systems (Alekshun and Levy 2007). Bacterial drug resistance thereby can be intrinsic due to the inherent property of the microorganism or acquired due to the evolutionary process of spontaneous mutations or gene uptake. Intrinsic mechanism makes bacterium naturally resistant to antibiotics like all mycoplasma withstand β-lactam action due to lack of peptidoglycan wall. A primary reason for resistance development is due to the spread of the antibiotic resistance genes on the plasmids that get multiplied independently and carried over to the next-generation doublets thereby conferring resistance (Yoneyama and Katsumata 2006). In certain cases, such genes can be evenly segregated onto the mobile genetic elements like transposons that can jump from one DNA locus into another. Similar occurrence happens in *Enterococcus*-resistant vancomycin strain where the five genes of microbial cargo hop into variant genomic locales. In medical environments, selective antibiotic pressures on pathogens like *S. aureus* and *Enterococcus faecalis* enable them to switch their antibiotic-susceptible profile to resistant ones. For instance, the methicillin-susceptible strain of *S. aureus* (MSSA) becomes resistant (MRSA) in patients with surgical treatments within a span of 5 days in hospitals. These isolates become resistant to vancomycin treatment. Hence, the antibiotic pressure alters the sensitivity genomic contour of the pathogen triggering the antibiotic resistance switch, for which new discoveries should target the molecular approach of resistance that makes these pathogens superbugs (Walsh 2000).

The major antibacterial drug resistance strategy involved is mostly the *efflux pump mechanism* where the microbe pumps out the antibiotic preventing their disruption of cellular processes (Poole 2005). The intrinsic mechanism of resistance is known to induce the expression of efflux pump systems. In *Pseudomonas aeruginosa*, the disruption of the functioning of MexB pump raises the sensitivity

to antibiotics like tetracyclines, chloramphenicol, β-lactams, etc. (Lomovskaya et al. 2001). Hence antibiotics can regulate the efflux pump expression in microbes at the transcription level of gene function. Transporters can be expressed due to the modulation of these regulators. In *Neisseria gonorrhoeae*, the efflux machinery encoded by the *mtr* operon eases the transport of antimicrobial components. However, mutation in the mtrR gene increases the bug's resistance to penicillins, macrolides, as well as rifamycins (Veal et al. 2002). Secondly in *P. aeruginosa*, exposure to fluoroquinolones leads to mutations in the genes encoding for efflux proteins as well as topoisomerases. Again mutation in efflux genes augments the resistance level to fluoroquinolones in *P. aeruginosa*. *Streptococcus pneumoniae* is known to be resistant mostly due to the drug efflux pumping out bacterial strategy. The resistance in *Streptococcus* occurs in a stepwise regulated way initiating in *parC* mutations, followed by *gyrA* and then PmrA pump (Jones et al. 2000; Piddock et al. 2002). Each level of mutation confers to a greater degree of resistance. In the 1980s, active efflux became major players in antibiotic resistance that led to the emergence of multiple drug-resistant strains (MDRs). Efflux transporters are known to be polyspecific where they eject out a huge diversity of structurally nonidentical components (Piddock 2006b). Additionally the MDR efflux systems eject out toxic compounds enabling the microbe to escape the classic antibiotic therapy (Paulsen 2003). There are five basic protein families that form the category of bacterial efflux pump system. Two well-known protein families are the ABC superfamily (ATP-binding cassette) along with the MFS (major facilitator superfamily). Other three small units include the SMR (small multidrug resistance), the RND (resistance–nodulation–cell division), and the MATE (multidrug and toxic compounds extrusion) family (Piddock 2006a). Other than ABC family pumps, the rest transporters are termed as secondary transporters conjugated to proton influx, hence called as H1-drug antiporters. Conversely, the primary transporters, i.e., ABC, utilize ATP for their activity.

5.2.2 Antibiotic Efflux Pump System

Antibiotics require a particular site of action and minimal level of concentration for targeting microbial cellular processes. For instance, if the antibiotics have to target the protein biosynthetic pathway, then the membrane barrier has to be surpassed for disrupting the protein assembly at high accumulated concentrations. The Gram (+ve) and the Gram (−ve) bacterial strains have overexpressed the active efflux pumps to expel out tetracycline to gain antibiotic tolerance (Nikaido 1996). A huge variety and diversity of export pumps are employed for a large spectrum of lipophilic or amphipathic compounds to keep the diffusion level low for unhindered bacterial sustenance. Some examples show the export pumps to be expressed by the antibiotic-producing cells for expelling out the antibacterial compounds as a protective shield to prevent the suicidal death of the microbe by its intrinsic weapon.

Efflux Transporters The most important factor in causing bacterial death is the accumulation of harsh antibiotics within the cytoplasmic fraction for inhibitory effects or growth attenuation. With Gram-positive bacteria lacking an outer plasma membrane barrier, the production of transporters enables the efflux mechanism to confer resistance within such species. Additionally the tough exterior of Gram-negative bacteria avoids drug buildup. As mentioned above, the Gram-positive bacteria comprise of three protein transporter superfamilies, namely, the ABC family of transporters which harnesses cytoplasmic ATP for antibiotic ejection and MF and SMR transporter protein groups which exploit an electrochemical proton gradient for efflux mechanism (Markham and Neyfakh 2001). The molecular mechanism of relating the ATP and proton exchange with drug efflux is still under investigation. Failures in X-ray structural characterization of transporter molecules have also raised major scientific concern. The examples of substrate-specific efflux pumps are either macrolide specific or tetracycline targeted (TetKLZ) which confer an additional function of immunity for the microbe. The issue of drug recognition can be resolved by getting an insight into the structural aspect of proteins regulating the efflux protein expression. The TetR protein binds to tetracycline leading to the overexpression of tetracycline substrate-specific transporter. This drug DNA-binding repressor undergoes a conventional van der Waals mode of interaction where the H bonds facilitate the linkage of tetracycline–Mg^{2+} complex with the polar amino acids bound with water molecules. The ligand binding occurs proper with a defined chemical architecture. So tetracycline transporters undergo substrate-specific interaction that governs their efflux mechanism. Another level of interaction occurs in the transcriptional regulator of the well-known bacterial species of *Bacillus subtilis*. The BmrR regulator induces the production of Bmr multidrug efflux protein which expels out intracellular hydrophobic cations (Paulsen 2003). In such case of regulators, the ligand binding occurs by means of both electrostatic and hydrophobic interactions rather than hydrogen bonds. So the dependency on structural outlook of substrate specificity becomes limited. The substrate specificity of the transporters is linked to its multidrug abilities. For instance, the evolutionarily distinct transporters of *B. subtilis* (Bmr, Blt) and *S. aureus* (NorA) share functional and sequence homology with transporters of *Lactococcus lactis* (LmrP) and *Staphylococcus* (QacA) itself as they belong to the identical MF family. They are much more homologous to the tetracycline transporters of Gram-negative bacteria. Even there is very insignificant identity among the same group of transporters like tetracycline, efflux proteins TetK and TetL bear very less similarity to TetZ of *C. glutamicum*. Both TetK and TetL share much similarity rather than the multidrug efflux units of *Staphylococcus* QacA. Another example to illustrate the mechanism of substrate recognition and binding is the class of lipocalins (Gunn 2008). These proteins share a similar tertiary structural outlook and bear high binding affinity for diversified substrates (ligands). Some lipocalins are even identical to multidrug efflux units enabling the organic compounds to interact with the hydrophobic protein core. The diversity of substrate binding by lipocalins was well demonstrated through mutagenesis of the hydrophobic binding residues. This resulted in switching the affinity partially

toward the hydrophilic fluorescein moieties. This led to the modulation of substrate recognition. Hereby, Gram-positive bacterial species bear different modulated transporters under the ABC, MF, and SMR family to force out the harsh antimicrobial agents.

There are around a dozen or two efflux membrane transporters in Gram-positive bacteria. One or more efflux transporters can also be present on the same microbe forming multiple drug transporters with an array of substrates. In *B. subtilis*, around four multidrug transporters have been reported, namely, Bmr, Bmr3, and Blt MF efflux systems (Ahmed et al. 1995), and one SMR family protein EbrAB. The sequence homologies screened many other hypothetical transporter genes in *Bacillus*. Efflux mechanism can be an opportunistic attempt by these multidrug transporters. For instance, tetracycline transporters have an additional function of transporting monovalent ions like Na^+ and K^+; other than tetracycline and genetic alterations of such transporters confers antibiotic and saline susceptibility. Examples of such transporters are TetL and TetK from *B. subtilis* and *S. aureus*, respectively. Being translationally regulated by tetracycline, these transporters channel the transport of monovalent ions as a side effect of drug transport. Similarly in *B. subtilis*, the two specific multidrug transporters Bmr and Blt are distinctly and differentially regulated at the transcriptional level (Ahmed et al. 1995). Blt and Bmr transporters extrude polyamines from the bacterium. However the Bmr–BmrR combination functions to assure toxin protection. Specific transporters when studied, their functional relevance for multiple purposes comes into significance.

The MLS class of antibiotics hinder protein synthesis by targeting the 50S ribosomal subunit. Other than target modulation and enzymatic blockage, the efflux pump mechanism in these antibiotic classes showcases tolerance features in Gram-positive strains. The efflux mechanism was due to *msrA* and *msrB* genes discovered in *Staphylococcus* and *msrC* gene in *Enterococcus* provided 200-fold and eightfold increase in resistance, respectively (Schmitz et al. 2000). In macrolide-resistant *Streptococcus*, the *mefA* or *mefE* genes augment tolerance level to 60-fold. These genes code for MF family transporters. The *mef* genes account for macrolide resistance in many Gram-positive as well as Gram-negative pathogens. It is present within mobile transposons; thereby, the tolerance mechanism will spread rapidly among other pathogenic species. Similarly, tetracycline transporters TetK and TetL predominantly account for antibiotic tolerance in *Staphylococcus* as well as *Enterococcus*. For greater antibiotic efficiency, decreased affinity of tetracycline derivatives via efflux pump mechanism has been approached, for example, glycyclines. The second approach encourages the use of tetracycline analogs in conjugation with the antibiotic blocking efflux transporters. Fluoroquinolone resistance occurs due to topoisomerase and DNA gyrase modulation by multidrug efflux transporters, for instance, NorA of *S. aureus* belonging to MF family. Greater NorA expression leads to acquired ciprofloxacin tolerance in addition to intrinsic fluoroquinolone resistance. Targeting efflux pump mechanisms increases the response of pathogen to antimicrobials as well as promotes drug accumulation within the bacterium. Cationic peptides target different resistance mechanism of *Pseudomonas* against

antimicrobials, namely, efflux method and target site modification to highlight the role of membrane barrier as a target for overcoming pathogen tolerance (Lin et al. 2010).

5.2.3 Degradation of Antibiotic

The second strategy involves degradation of the chemically active component of the antibiotic weapon. Accumulation or expelling out antibacterial agents from cells doesn't modulate with the structure of the antibiotic. Like in penicillins and cephalosporins, the inactivation of β-lactam ring will cripple the efficacy of the antibiotic itself. B-lactamase is the enzyme that catalyzes this modulation. The active ring enables acetylation and modulation of the peptidoglycan cross-links, whose disruption renders the antibiotic nonfunctional. The lactamase enzyme is produced in the bacterial periplasm to inactivate the cytoplasmic antibiotic targets. A single enzyme can cripple about hundred penicillin particles, so the greater the enzyme, the higher the intensity of antibiotic destruction and the more efficient the strategy. However, other antibacterial compounds like aminoglycosides aren't prone to such hydrolytic cleavage. Aminoglycosides target the protein synthetic machinery and bear three specific chemical alternates that bring modulation in the ribosomal RNA binding. The aminoglycoside resistance can be due to the adenylyl, phosphoryl, or acetyl transferases which insert either an AMP moiety or phosphate group or bring about amino acid acetylation. These modifications decrease the RNA binding affinity and disrupt with the protein synthesis. The structural elucidation of phosphotransferase shows a direct evidence of evolutionary link to kinase enzyme thereby facilitating the recruitment of bacterial resistance strategies.

5.2.4 Alteration of Bacterial Target

The third resistance approach employed by the microbes involves the modulation or reprogramming of the target enzyme in the resistant pathogen. This camouflage mechanism can occur in conjugation with the efflux mechanism thereby adding up to the resistance strategy. For example, the erythromycin-resistant strains alter the adenine moieties by methylation in the peptidyl transferase loop of ribosomal RNA unit (McCusker and Fujimori 2012). The erythromycin ribosomal methylase gene targets the decreased RNA affinity for erythromycin as well as pristinamycin drugs without blocking the protein synthesis. This method of methylation is the prime machinery of resistance in the virulent species of *S. aureus* and acts as immunity armor against erythromycin-expressing strains. Other than erythromycin, target modulation is also observed in vancomycin-resistant enterococci (VRE) to escape the harsh antibiotic effects. In the resistant *Enterococcus*, the *vanHAX* gene encodes a pathway where these three genes play different modulatory roles for providing a survival advantage to the bacterium (Sood et al. 2008). *vanH* gene enables pyruvate reduction to D-lactate followed by *vanA* forming D-Ala-D-Lac and *vanX* hydrolyzing

the D-alanine dipeptide rather than D-Ala-D-Lac linkage. Overall, the accumulation of D-Ala-D-Lac substrate becomes the point of elongation and extension at the peptidoglycan terminal strands. This remodulation of the D-alanine dipeptide to D-Ala-D-Lac affects the degree of vancomycin binding by 1000-folds without impairing the glycan and peptide cross-linking efficiency. This tolerance confers a greater profile of vancomycin resistance. Consequently not only does β-lactamase production affect the resistance mechanism, but also the penicillin-binding proteins bring about lower antibiotic affinity. In *Staphylococcus aureus*, the characterization of a penicillin-binding protein encoded by the *mecA* gene will help to elucidate the molecular mechanism of MRSA phenotype.

5.3 Tuberculosis: The Unsolved Puzzle

The last millennium has witnessed the generations of antibiotic development with the startling increase in resistance against those antibacterial drugs. Among the life-threatening species, *Mycobacterium tuberculosis* has become a global threat for mortality with time. The major survival advantage of *M. tuberculosis* is its dormant stage which sustains in asymptomatic hosts that later on leads to disease. *Mycobacterium tuberculosis* makes use of all the efflux transporters for their survival. The major two mechanisms thought to play a pivotal role in mycobacterial drug resistance are the cell wall barrier and the efflux pump machinery (Silva and Palomino 2011). The genes that encode for efflux transporters have been extensively studied as they encode for proteins that channelize compounds like tetracycline, fluoroquinolones, aminoglycosides, and drugs like isoniazid used for tuberculosis treatment itself. Hence, the balance between pumping out antibiotics and enabling cellular drug intake is yet to be explored further for new inventions (De Rossi et al. 2006). In spite of the BCG vaccine, resistance has also been observed against many anti-TB compounds. With the course of time, the *M. tuberculosis* strains have developed mutations that have actively targeted the drug stimulation giving rise to MDR-TB strains. For instance, the streptomycin resistance develops due to changes in the genes like *rrs* and *rpsL* which alters the ribosomal binding site for streptomycin. Even the *pncA* gene alteration leads to pyrazinamidase resistance in this notorious bug. Isoniazid has drug targets that are involved in the cell wall biosynthesis (mycolic acid), but they don't entirely confer resistance. The prime reason of natural resistance in *M. tuberculosis* is decreased cell wall permeability due to high lipid content which limits cellular drug intake. Another factor that comes into play is the efflux system that forces out antimicrobial agents. The microbial efflux and influx balance thereby contributes to the microbial sustenance.

5.4 Antifungal Drug Resistance

The evolutionary problem of resistance has become well documented with time. Antifungal drug resistance also isn't exempted from such threat, though antibacterial resistance is a greater concern. The microbial world encompasses various carriers of infections among which fungal pathogens flourish in adaptable population. Under the administration of antifungal agents, the sensitive fungal lot evolves resistance mechanism against the drugs. The era of the 1980s had a very thorough study on the biochemical, genetic, and clinical aspect of antifungal resistance, but presently the elucidation of cellular and molecular mechanisms is under investigation (Anderson 2005). The drug targets are mostly designed for the fungus and less for the host, so a depth of understanding into the molecular mechanism of antifungal resistance can promote a divergent long-term host survivability. However the pathogen fitness on environmental impact and evolution of potential mutations causing divergent resistance can be explored further with experimentation. The strategy implementation for combating drug resistance would require hindering with the pathogen's evolutionary sustenance approach. A study of mutation, pathogen fitness, and multiresistant factor interacting in combination for a collective phenotype can undoubtedly modulate with the pathogen's gene expression and help us reduce the chances of increased drug resistance. Greater incidence of resistant fungal pathogens has increased the risk factor of mortality in patients bearing severe immunosuppression. Though novel drugs have come up, still patients under long-term antifungal medication undergo a microflora transition during the course of time which further leads to the development of an apparent resistance mechanism.

The nineteenth century had witnessed drug resistance problem pertaining to a range of infectious diseases like tuberculosis, salmonellosis, HIV, etc. The scientific world also came across the problem of fungal infections during that time which posed a threat to health and life. This was mainly due to a change in the immune profile of the patients who were inflicted with AIDS or cancer or had undergone any sort of transplantation. That time demanded the urgent requirement of new invention of antifungal drugs as compared to the conventional ones with least side effects and with more impact on combating infections rather than being resistant to the new emerging pathogens. One study reported 33 % of patients with AIDS did bear resistance against *Candida albicans* (Sanglard et al. 1995). About 200 out of the 1.5 million species within the fungal kingdom are associated with human diseases. Some are commensals, whereas others like *Candida* are opportunistic species which infect when the host's immune system cripples down. Though skin infections are initial symptoms, systemic fungal infections causing dissemination are difficult for diagnosis and cause greater incidence of mortality. The epidemiological survey lists *Candida*, *Aspergillus*, and *Cryptococcus* species to be the causative agents of infection-related mortality. Mostly azole antifungals are used to treat infections and fluconazole usage decreases *Aspergillus* infection from 10 % to 20 %. However

azole resistance has emerged due to acquired mechanism in the opportunistic species or due to selection pressure in the innate resistant strains.

The drug resistance mechanism among the fungal pathogens is mostly due to the reason of increased efflux where there happens to be an overexpression of certain transporters of cell membrane (Cannon et al. 2009). This mutational change in the transcriptional regulator confers a resistance characteristic. Secondly, an alteration in the protein target causes either a change in antifungal drug binding or allosteric inactivation of the enzyme. Some minor changes in amino acid sequences bestow the pathogen with such resistant phenotype by altering the drug activity. However, higher extent of amino acid alteration leads to functional loss of protein and accumulation of unwanted products called toxin with no significance of the drug. Thereby, altering metabolism also confers resistance mechanism. The standardized measurement of antifungal drug resistance is with the protocol of minimum inhibitory concentration (MIC) where the terms "drug sensitive" and "drug resistant" are outlined. However when MIC is not clear during growth transition, parameters of fitness analysis can be used to quantify resistance. At times, tolerance assays can be used for lethal drug measurements though it's not positively related to drug resistance.

The resistance in microbial population is due to evolutionary processes. However in a mixture of resistant and sensitive strains, the phenotype of resistance is not clearly defined. The population size and mutational effects confer drug resistance within the eukaryotic environment. With high incidence of opportunistic fungal pathogens, the immunocompromised individuals fall into the trap of mortality of these invasive species. *Candida* and *Aspergillus* being the most threatening species of concern are the cause of death rate of about 40–50 %. The antifungal resistance has mainly risen up due to triazole drugs that have conferred both primary as well as secondary resistance with apparent shift of colonization markers in the susceptible strains. Triazoles like fluconazole, posaconazole, etc., are mostly used to treat *Candida* infections. A study done over a decade showed 140,000 *Candida* strains to be resistant to fluconazole and voriconazole by 6 % and 3 %, respectively (Pfaller et al. 2010). The Netherlands and UK reported triazole resistance to shoot up to sixfolds over a period of 14 years. This section will outline the details of antifungal drug resistance mechanism and the strategies to combat such problems in the future.

5.4.1 Antifungal Agents and Their Mechanism of Action

The mechanism of action of different antifungal drugs is an essential prerequisite for getting an insight into their resistance mechanism. The choice of the antifungal drug should be based on factors concerning the host specificities and drug properties like its absorption and toxicity features (Odds et al. 2003). The host immune profile, the pathogen specificities (i.e., fungal species and its response to drugs) affecting the site of infection, and the pharmacokinetic properties of the antifungal agent should be taken into consideration. Very less antimycotic agents have been used to treat systemic infections. According to the action mechanism, the antifungal

drugs are categorized into four different classes, namely, polyenes, azoles, nucleic acid synthesis inhibitors, and inhibitors of glucan synthesis. Their mode of action enabled a clear understanding and elucidation of their resistance mechanism. Out of these, three antimycotic agents, namely, polyenes, azoles, and allylamines, have their antifungal effect due to their inhibitory property on synthesis/interaction of ergosterol, a major fungal membrane component.

5.4.2 Ergosterol Biosynthesis Inhibitors (Azoles and Triazoles)

The 1970s witnessed the discovery of azoles, clotrimazole being the first azole-based drugs for systemic infections. These N-substituted imidazoles are compounds ranging from miconazole to ketoconazole and fluconazole. Due to certain limitations of miconazole, ketoconazole became the first commercialized oral antifungal medication against chronic candidiasis (Petersen et al. 1980), with an exception to *C. glabrata*. For human use, itraconazole and fluconazole were the triazoles for oral as well as intravenous administration. The safety and efficiency of fluconazole has been clinically approved for global use. Itraconazole was used against *Candida* spp. as well as *Aspergillus* spp. (Pfaller et al. 2005). Similarly, fluconazole intake could decrease invasive candidiasis in patients undergoing chemotherapy or transplantations. The frequent use of fluconazole has led to a resistant host microflora against the medication. The twentieth century witnessed the clinical approval of voriconazole for global use in the USA. Additionally, two other triazoles, namely, posaconazole and ravuconazole, were scrutinized for their action and efficacy against *Candida* species. Voriconazole, structurally and functionally similar to fluconazole and itraconazole, was used in conjugation with liposomal AmB for medication. Posaconazole was effective against *Candida* spp., *Aspergillus*, as well as *Cryptococcus*. From experimentation, posaconazole was found to be the most efficient triazole against itraconazole-sensitive strains. Ravuconazole also showed additional effect on *Fusarium*, histoplasma, *Blastomyces*, etc. The triazole drugs differ in their mechanism of action, resistance, as well as cross-resistance pattern among microbes. For instance, a voriconazole-resistant *Aspergillus* isolate has slight cross-resistance among itraconazole, posaconazole, as well as voriconazole. Similarly for *Scedosporium* strains, no cross-resistance was observed among triazole drugs like miconazole, itraconazole, or voriconazole. Neither was any sort of resistance reported against posaconazole medication.

The plasma membrane bioregulator ergosterol maintains the fungal cell integrity. The demethylation of lanosterol is catalyzed by 14a-demethylase in a cytochrome P-450-dependent manner. Alteration of this target enzyme results in structural and functional modulation of fungal membrane. Azoles inhibit the synthesis of ergosterol, an essential fungal membrane component by blocking the activity of the enzyme lanosterol demethylase which catalyzes the reaction of ergosterol biosynthetic pathway (Bossche 1985). The heme domain of the enzyme is bound with the nitrogen atom of azole ring to prevent lanosterol's demethylation. Azoles also target methylsterol synthetic pathway. Azole resistance is prevalent in

patients with HIV infections which undergo long-term treatment procedures to combat mucosal or oral *Candida* colonization (Lupetti et al. 2002). This frequency of azole resistance has markedly increased with the course of time. With the amount of CD4 cells, pathogen load, and therapy dosage, the incidence of resistance varies. A study reported the presence of resistant *C. neoformans* from a healthy patient without having any previous fluconazole medication (Orni-Wasserlauf et al. 1999). Another study reported the HIV-infected patients bearing *C. albicans* infection to be resistant to clotrimazole (Pelletier et al. 2000). Certain cases also witness a profile of cross-resistance (Müller et al. 2000). Many species of *Candida* like *C. krusei* have an intrinsic resistant characteristic to fluconazole which is prevalent in patients infected with HIV, cancer, or undergoing transplantation. Azole antimycotic drugs have an array of heterogeneous functions ranging from acting as inhibitors of membrane-bound enzymes to the blockage of lipid biosynthetic pathway. Furthermore, azoles like fluconazole and itraconazole bring about the aggregation of sterol precursors in *Cryptococcus* by the reducing obtusifolione. Even the demethylation affects the cholesterol synthesis in mammals by a greater dosage of azoles. A study done by Hitchcock et al. reported that 50 % inhibitory concentration of voriconazole had 250-fold more activity against the mammalian demethylase as compared to the fungal enzyme (Martin et al. 1997). So azoles have their action to be genus based.

Azole resistance mechanisms are mostly similar to antibacterial mechanism like the target enzyme modification, the efflux pump resistance mechanism, and the aminoglycoside tolerance with membrane alterations. The specificity against azoles is still a question as cross-resistance among this class of drugs is quite common. Bacterial strains have best evolved with efflux pumps for resistance mechanism like the ***mar*** (multiple antibiotic resistance) genes in *E. coli* (Cohen et al. 1993; Alekshun and Levy 1999). These genes are also associated for chloramphenicol and tetracycline resistance. These multidrug efflux pumps have also conferred resistance in *S. aureus* and *P. aeruginosa* against fluoroquinolones and β-lactams, respectively. The phospholipid and fatty acid content influence the membrane permeability as well as miconazole resistance in *C. albicans*. Similarly, in *P. aeruginosa* diminished membrane D2 porin expression as well as enhanced amphotericin β-lactamase expression enables imipenem resistance. No mutation in the gene; just the membrane composition can alter the microbe tolerance levels. Vancomycin's size exclusion by the bacterial membrane is another factor for its resistance.

Alteration of Drug Efflux Efflux pump machinery is mostly responsible for the dominance of resistance mechanism among the fungal pathogens. This is a common mechanism of antibiotic resistance in *S. pneumoniae*. The two efflux pumps in pathogens conferring azole resistance comprise of proteins belonging to the major facilitator superfamily (**MFS**) and ATP-binding cassette (**ABC**) superfamily. Moreover, ABC (ATP-binding cassette) transporters are the major culprits of drug resistance. MFS protein pumps involve the passage of structurally diverse components. MDR1 in fluconazole-resistant *Candida* strains were known to encode

resistance for benomyl as well as methotrexate. The ABC transporters require ATP for substrate channelization for which they bear two ATP-binding cytoplasmic moieties. Other than that, there are four core integral domains that span the membrane a couple of times. In *S. cerevisiae*, the ABC transporters recognized are classified into MDR, CFTR, YEF, and PDR families. Five CDR (*Candida* drug resistance) genes in *Candida* are responsible for azole resistance (White et al. 2002). CDR1 present in *Cryptococcus* and *Candida* is structurally similar to human P-glycoprotein. When *C. albicans* was experimented for its ability to mount up fluconazole, an overexpression of CDR1 levels was found with reduced drug concentrations. Secondly, reduced fluconazole accumulation could be due to the ATP-dependent drug efflux mechanism. A study by Sanglard et al. carried out experiments involving 16 *C. albicans* clinical isolates from five individuals with HIV infections (Sanglard et al. 1995). The aim was to observe the accumulation of fluconazole with treatment. Fewer amounts of fluconazole levels did correspond to tenfold higher CDR1 mRNA profile. However, with overexpressed CaMDR1 mRNA level, normal CDR1 transcriptomic levels were observed. This concludes that CDR1 is mostly involved in the transport of azole antifungals, whereas CaMDR1 gene specifically enables fluconazole resistance development. A CDR1 mutation enabled greater susceptibility of *C. albicans* to triazoles. Similarly, CDR2 induction resulted in azole resistance. A double knockout mutant (CDR1 and CDR2 deletion) strain showed greater susceptibility than a single gene disruption. Moreover using membrane potential as the driving force, MDR1 overexpression also leads to azole resistance. A study reported the accumulation of a fluorescent rhodamine 123 dye in *C. albicans* and *C. glabrata* (Clark et al. 1996). This dye is specifically transported by the MDR machinery. This mechanism also leads to a phenomenon of efflux competition. Additionally, ABC transporters overexpression is a crucial factor in promoting azole resistance in *C. glabrata* isolates. However, exposure to azoles can contribute to a transcriptional alteration in CDR profiling. The sterol composition doesn't influence resistance; rather the genes like ERG16, MDR1, and CDR1 are involved in microbial tolerance mechanism. Secondly continuous azole exposure can lead to an induced expression of *ERG16* as well as *CDR1* genes, thereby leading to cross-resistance among other azole medications.

Alteration of the Target Enzyme The most common example with respect to enzyme alteration is lanosterol demethylase whose overexpression confers azole resistance in *C. glabrata*. The modification of this enzyme plays a pivotal role in conferring azole resistance mechanism. No difference in sterol distribution was found in two fluconazole resistant and susceptible *C. krusei* strains. The inhibitory effect of fluconazole was 20–40 % higher in *C. krusei* than *C. albicans*. This is due to greater active efflux mechanisms in *C. krusei* isolates. With respect to 14a-demethylase enzyme modification, the azole-susceptible units were found to have an altered peak in the carbon monoxide spectra of the cytochrome. Moreover, this enzyme has low affinity for azole drugs. Whether alteration of the enzyme is the sole factor remains still a question. Thereby, certain cases have reported the overexpression as major criteria for resistance mechanism. The overexpression

leads to enhanced copies of the enzyme which promotes ergosterol synthesis and enables resistance development against fluconazole as well as itraconazole. Here the ergosterol increase could be attributed to the overexpressed enzyme as well as to the less susceptibility of both azoles and amphotericin B. Some studies have focused on P-450 levels for conferring cross-resistance in azoles. But in *C. albicans*, the increased expression doesn't have much of an impact. Azoles are inhibitors of ergosterol synthesis by modulating the binding with the demethylase enzyme. Further the *ERG11* gene was investigated for its role in drug selection pressure. Sequence analysis in *C. albicans* targeted amino acid substitutions at the active site of the enzyme (i.e., the heme domain) to be responsible for resistance development. *ERG11* (*ERG16*) is the gene encoding for the protein, also termed as CYP51A1 in *C. albicans* (Marichal et al. 1999). Any alteration or mutation in this gene conferred azole tolerance. Lysine substitution with arginine at 467th residue near the heme domain brought about functional alteration to the enzymatic activity. Even a 464th residue substitution in *C. albicans* caused heme domain alteration contributing to fluconazole resistance with reduced activity. A study reported a T315A substitution in *C. albicans* showed twofold reduction in lanosterol demethylase catalytic activity and diminished affinity for fluconazole (Lamb et al. 1997). These substitutions also lead to decreased accessibility of the enzyme active site as was observed when the 105th phenylalanine residue was replaced with leucine in *C. albicans*. The antifungal agent fluconazole has a channelized entry into the active site where certain point mutations alter the accessibility of the substrates. These mutations are huddled up around three specific regions that are linked with resistance.

Alteration of the *ERG3* Genes *ERG3* gene encodes for sterol D(5,6) desaturase and an alteration or mutation in this gene confers azole resistance. In sensitive species, the azole exposure enables 14-methyl-3,6-diol accumulation causing a fungistatic property, however the mutation in *ERG3* gene together with the accumulation of the precursor (14-methylfecosterol) promotes fungal growth. *S. cerevisiae* has shown to exhibit azole resistance due to *ERG3* gene mutation (Martel et al. 2010). Some clinical isolates of HIV patients bearing resistant *C. albicans* showed an amassing of 14-methylfecosterol due to an impaired sterol D(5,6) desaturase. This impairment has also led to azole resistance in the fungi *U. maydis* (Joseph-home et al. 1995).

Alteration of the Drug Influx The drug uptake can be influenced by the plasma membrane composition and fluidity; for instance, the sterol content can affect the cellular drug influx. An alteration in the cell membrane constituents of *C. albicans*, like the phospholipids as well as the fatty acid content, can confer resistance to miconazole. A study by Hitchcock and Whittle reported a greater lipid profile and less polar to neutral lipid content in *C. albicans*-resistant strain as compared to wild-type one (Hitchcock and Whittle 1993). This hints reduced membrane permeability and decreased azole intake. Some reports demonstrated the fluconazole-resistant *Candida albicans* species to have reduced amount of ergosterol as well as low phosphatidylcholine: phosphatidylethanolamine profile (Löffler et al. 2000). Such

phenomenon modulates with the fluconazole uptake and intracellular accumulation. *C. krusei* also had decreased itraconazole accumulation rather than modulations in drug efflux mechanism or alteration in ergosterol amounts.

A study observed 20 isolates from HIV patients with oropharyngeal candidiasis (both susceptible and resistant to fluconazole) previously treated with azoles for the resistance frequency (Perea et al. 2001). About 85 % of those resistant strains had overexpression of drug efflux pumps with similar expression profile of CDRs and *MDR1* gene. Around 60 % isolates showed alteration in the lanosterol demethylase enzyme with 35 % having an overexpression of the gene encoding the enzyme. Around 75 % of these strains showed multiple mechanisms of resistance. Another study reported only overexpression of CDR genes in resistant strains without any correlation of tolerance to amino acid substitution or *MDR1*, *ERG11* expression profile (Marr et al. 1998). CDR-encoded pumps (*CDR1* and *CDR2*) play a regulatory role in conferring azole resistance mechanism.

5.4.3 Polyenes

Action and Resistance Mechanism From the 1950s, polyenes (AmB) have been known to be the standard treatment for systemic fungal infections. Amphotericin B, the broad-spectrum antifungal agent, targets ergosterol and is active against *Candida* species, *Cryptococcus neoformans*, strains of *Aspergillus*, *Zygomycetes*, etc. (Brajtburg et al. 1990). The polyene-susceptible pathogens are known to bear sterols in their cell membrane as compared to the resistant ones. The literature suggests sterol addition for counterbalancing the fungal inhibitory effect of polyenes. The physicochemical interplay between membrane sterols and polyene antifungals restricts the binding affinity of drugs. Their interaction can be directly quantified with UV absorbance. The mode of action of amphotericin B involves the aggregation of 8–10 polyene molecules to form a porin channel within bilayer membrane for disrupting fungal ionic gradient by loss of potassium ions. This pore enables polyene hydroxyl moieties to protrude inward thereby causing altered permeability and loss of essential cytosolic components. The fatty acyl components also render polyene antifungal susceptibility in yeast. Additionally, amphotericin B also acts as an oxidative load on fungal membranes thereby causing the death of *C. albicans*. At higher doses, polyenes also hamper the functioning of fungal chitin synthase enzyme. For the unpleasant activity of AmB, the research developed conjugated AmB with liposomes for better functioning in host and least toxicity. Some examples include Amphotec, AmBisome, etc. During liposome and AmB formulation, selective transfer method enables the AmB's transfer from donor liposome to target the membranal ergosterol facilitated by phospholipases (either pathogen or host) (Boswell et al. 1998). A formulation of liposomal nystatin and polyene Nyotran is under clinical trials for evaluation.

Primary resistance to amphotericin B has been reported in the isolates of *Candida* like *C. lipolytica*, *Candida lusitaniae*, etc. Intrinsic resistance has been

observed on *Trichosporon beigelii*, *Pseudallescheria* spp., as well as *Scopulariopsis* spp. Secondary resistance against polyenes has been described in *C. neoformans* and many species of *Candida*. Some hypotheses have led to the conclusion that initial medications with azoles can lead to amphotericin B resistance. The resistance mechanism can be either due to the category of sterol or the fungal membrane composition. In one study, cross-resistance was observed to amphotericin B in fluconazole-resistant *Candida* strains from HIV-infected individuals due to modulation in ergosterol synthesis (Heinic et al. 1993). The same isolates also showed cross-resistance to nystatin. Such polyene (AmB as well as nystatin) resistance has also been observed in trauma patients with compromised immune profile. The lipid complexes with AmB have the active amphotericin moiety released from tissue lipases in vivo. This confers resistance to AmB contributing to less drug efficacy but with lipid formulations; the edge shifts to increased efficacy and uptake during drug administration. An alteration in the membrane's lipid composition due to deficit ergosterol amount lowers the affinity of amphotericin B for binding to plasma membrane. However, the resistance is conferred majorly due to ergosterol and not due to altered sterol composition. Another factor for resistance mechanism is deposition of β-1,3 glucans in the pathogen's cell wall which enables greater access of larger molecules to membrane due to high cell wall stability.

Polyene tolerance develops by selection pressure when certain resistant isolates multiply naturally within small number of population. These naturally developed resistant strains produce altered sterols for binding nystatin at decreased affinity. The binding of nystatin to sterol influences cell membrane damage. With greater affinity, the membrane damage increases. Generally, the resistant strain is expected to grow slow as compared to the susceptible one. Polyene tolerance is lost after certain passages in media devoid of nystatin. However, sterol contents result in increased affinity for nystatin. So it's mutation that plays a role in tolerance development rather than selection. Polyene resistance is mostly studied using cells grown in increasing or gradient concentration of antifungals that generates mutants. The biochemical hypothesis outlines resistance to be a qualitative as well as quantitative factor of sterol content in cells. Altered sterol content decreases the binding of polyenes in resistant cells either due to complete lack of cellular ergosterol amount without associated change in overall sterol composition or by replacing the higher affinity polyene-binding sterols with less binding ones. At times steric modification or thermodynamic alteration in polyene binding can also lead to the resistance mechanism. However the major reason of tolerance due to decreased ergosterol content is not caused due to enzymatic degradation but blockage on synthetic pathway which leads to decreased polyene susceptibility. However it was reported that certain *Candida* strains possessed certain key sterols that enabled the resistance to polyenes (Hamilton-Miller 1972). D8 sterols possessing strains are more resistant to polyenes than the ones bearing D7 sterols.

In one study, AmB resistance in *Leishmania* species was attributed to ergosterol substitution in the membrane which alters the membrane fluidity and binding of amphotericin B (Mbongo et al. 1998). The interaction of polyenes with the

membrane components have been extensively studied and explored by researchers. Stationary phase cells are more prone to polyene resistance as compared to their exponential counterparts because of less active cells involved in synthesis of cell wall components during static phase. So the access to membrane slows down. Thereby drug modulation can't influence polyene tolerance mechanism. Efflux pump method can't be involved in polyene resistance development. The genetic basis of polyene resistance in *S. cerevisiae* is related to the mutations in *pol* genes (Molzahn and Woods 1972). The mutants had a decreased amount or complete lack of ergosterol which establishes the much talked about antifungal resistance mechanism.

Allylamines The functional and chemical aspect of allylamines makes them distinct from the group of ergosterol biosynthetic inhibitors. These antifungal agents like terbinafine are effective against dermatophytes and azole-resistant *Candida* as well as *Cryptococcus* species. Squalene accumulation during ergosterol biosynthetic pathway at the step of squalene epoxidation is the direct target of allylamine inhibition (Ryder 1992). Squalene epoxidase is essential for allylamine activity, thereby hinting fungal death due to higher levels of squalene rather than ergosterol deficiency. Accumulation of squalene leads to greater membrane permeability and obstruction to cellular organization. However, extensive usage of terbinafine and naftifine can confer cross-tolerance to fluconazole-resistant *Candida* strains. Azoles, polyenes, and allylamines play similar mechanism of action targeting cell wall synthesis like penicillin, vancomycin, and other antibacterial agents.

5.4.4 5-Fluorocytosine: Nucleic Acid Synthesis Inhibitors

Action and Resistance Mechanism A fluorinated pyrimidine fluorocytosine has been prevalent for use against fungal infections since the era of 1960. With an efficient penetrating capability into body fluids, 5-FC was targeted against *Candida* as well as *C. neoformans*. However, the incidence of primary resistance among fungal pathogens led to the combinational therapy of 5-FC along with other antifungal drugs like amphotericin B along with fluconazole. The cytosine permease enzyme aids the absorption of 5-FC into the fungal cells (Andriole 1999). Immediate to its entry, the compound undergoes modification to 5-fluorouracil by deaminase enzyme. This compound undergoes conversion to fluorouridine triphosphate by UMP pyrophosphorylase which after further phosphorylation is incorporated into the fungal RNA chain, thereby blocking the protein synthesis. Fluorouracil also undergoes conversion to 5-fluorodeoxyuridine monophosphate which disrupts with the functioning of thymidylate synthase, which aids in DNA synthesis. Thereby, 5-FC disrupts with DNA, RNA, as well as protein synthesis of pathogen cell. The resistance mechanism have been widely investigated and hence have related the disrupted functioning of the enzyme cytosine permease or deaminase (which enables two consecutive absorption and conversion steps of 5-FC) to

diminished drug uptake by fungal cells. This is due to a mutational change that not only promotes primary but also intrinsic resistance. Secondly, less catalytic activity of uridine monophosphate pyrophosphorylase and uracil phosphoribosyltransferase also confers 5-FC resistance in *C. albicans*. Both cytosine deaminase and uracil phosphoribosyltransferase are involved in the pyrimidine salvage pathway and are unessential for de novo synthesis. A change in the bases due to mutation in either one of these salvage pathway enzymes confers resistance in *C. albicans* and *Cryptococcus neoformans*. In *C. albicans*, less phosphoribosyltransferase activity was linked to 5-FC resistance in a dose-dependent manner. Resistance to 5-FC due to decreased uptake has been well observed in *S. cerevisiae* and *C. glabrata* (Vandevelde et al. 1972). However, this mechanism is not of significance in *C. albicans* or *Cryptococcus neoformans*.

5.4.5 Inhibitors of Glucan Synthesis: Fungal Cell Wall Compounds

Mode of Action and Resistance Mechanism The multilayer fungal cell wall consists of unique components like α- and β-glucans, mannan, chitin, etc., which provide antifungal drug targets for safe use in mammalian hosts. These compounds have selective toxicity benefits in host. The medically important pathogen *C. albicans* has a multilayered cell wall comprising of β-glucan and mannoprotein (80 % of cell mass) along with chitin. Among the glucan synthesis inhibitors, chitin biosynthetic genes are disrupted by compounds named as nikkomycins which have been scientifically investigated for commercial use. The three groups categorized as glucan synthesis inhibitors are aculeacins, echinocandins, and papulacandins which disrupt the functioning of β-(1,3)-glucan synthetase enzyme that leads to synthesis of 1,3-β, D-glucan. However among the inhibitors, the lipoproteins, i.e., echinocandins, are clinically approved for global use due to their safety, efficacy, and tolerance level. Echinocandins are tested to be potently active against *Candida* spp., *Aspergillus* strains, dimorphic molds, and *Pneumocystis carinii*. Three antimycotic compounds under echinocandins have been thoroughly investigated for safe use, namely, caspofungin, FK-463, and VER-002. No cross-resistance was observed against these three compounds in isolates resistant to triazoles. These inhibitors form a noncompetitive inhibition with secondary effects on either the chitin content or ergosterol content of fungal cell. One study reported the in vitro comparative activity analysis of echinocandin in fluconazole-resistant *Candida* strains with itraconazole and amphotericin B as test parameters (Cuenca-Estrella et al. 2000). The results showed echinocandin to have potent activity against a range of *Candida* spp. like *C. albicans*, *C. tropicalis*, *C. krusei*, etc. However, echinocandins showed to have less activity against *C. parapsilosis* and *C. guilliermondii*. Nevertheless, in *C. neoformans*, the reduced activity of echinocandins is due to decreased fungal glucan synthase activity (Maligie and Selitrennikoff 2005). The resistance mechanism of pathogens against echinocandin is very limited. Kurtz and Douglas experimented with the laboratory-resistant mutants of *S. cerevisiae*. Echinocandins target the β-glucan synthase enzyme

which is mainly encoded by two genes called *FKS1* and *RHO1* and regulated by a third gene (Sekiya-Kawasaki et al. 2002). FKS2 is another gene in S. *cerevisiae* which is homologous to *FKS1*. Any base alteration in *FKS1* genetically causing mutations leads to the development of in vitro resistance to echinocandins (Balashov et al. 2006). This is specifically due to the alteration of the target enzyme, glucan synthase. Some sort of resistance is also developed due to mutations in *GNS1* gene coding for fatty acid chain synthesis in cell wall. Conversely, changes in *FKS2* gene does not attribute to resistance. The resistance mechanisms for azoles include efflux pumps and fungal membrane composition, which howsoever seems inappropriate for echinocandins as they don't undergo cytosolic pathway of penetration. Since they don't traverse the fungal membrane barrier, the entry mechanisms don't serve as the methods of resistance development in lipopeptides. Even MDR-like gene activation doesn't confer resistance mechanism to echinocandins. These results finally conclude that *FKS1* mutation can alter the protein forming the catalytic target of glucan synthase enzyme, thereby facilitating lipopeptide resistance development in *S. cerevisiae*. Lastly, a study established that both *S. cerevisiae* and *C. albicans* have similar mechanism of resistance to β-glucan synthase inhibitors (Douglas et al. 1994).

5.5 Antiviral Drug Resistance

In the late twentieth century, the development of potent antiviral drugs was considered as an important achievement in the field of biomedical science. Highly effective drugs against a wide range of viruses like herpes, HIV, hepatitis B, influenza, human papillomavirus, respiratory viruses, enteroviruses, hepatitis C, etc., have been designed and proved to be of human benefit. But sadly, with time, antiviral drug resistance has emerged at a considerably higher rate. The resistance to antiviral agents is considered to be a natural phenomenon because of the rapid replication ability of the virus under a selective pressure (Richman 2006). On prolonged drug exposure only those mutants survive which can replicate continuously in that environment and thus become resistant. The development of resistance is a major point of concern in the immunocompromised patients too (Strasfeld and Chou 2010). Quick diagnosis of the resistance type can be made by observing the different mutations in the genome of the viruses that made them resistant. A lot of literatures describe drug resistance in influenza virus, retroviruses (HIV), herpes simplex virus, cytomegalovirus, varicella-zoster virus, and hepatitis B virus (discussed in detail below).

5.5.1 Drug-Resistant Influenza Virus

Adamantanes like amantadine and rimantadine are drugs mainly given for treating influenza A viral infections. But clinical studies have shown this virus to be

increasingly resistant to both the drugs, especially amantadine in both animal and human isolates (Englund et al. 1998). The drug-resistant strains showed point mutations in a recognized second reading frame of the M segment of the influenza RNA genome. This region, named as M2 encodes for a tetrameric, transmembrane H^+ ion channel, required for pH mediated entry of the viral ribonucleoprotein into the cytoplasm. Amantadines used to block this channel, hence preventing viral replication. Early experiments with viral neuraminidase inhibitory drugs like oseltamivir and zanamivir showed sensitivity toward both influenza A and B viruses, but recent reports show incidence of higher resistance for oseltamivir than zanamivir. A seasonal influenza H1N1 virus found to be mutated in its neuraminidase gene (H274Y) and thus contribute for developing resistance against oseltamivir (Control and Prevention 2009). Some isolates of avian influenza A virus (H5N1) have also been adamantane resistant.

5.5.2 Drug-Resistant Herpes Simplex Virus

Mucocutaneous HSV infections are usually administered with acyclovir, valacyclovir, and famciclovir, whereas the first is always preferred in the treatment of serious invasive disease like encephalitis. Drug-resistant acyclovir was first encountered as early as in the year 1982 when the drug was systemically circulated, but later drug-resistant strains have been isolated from patients without any history of preexposure to the drug (Nugier et al. 1992). Resistance of HSV to acyclovir is often associated with the viral TK or DNA polymerase mutations (Morfin and Thouvenot 2003). This mutation can lead to a loss of TK function or a modification in TK substrate specificity. Mutations in the TK gene are mainly due to addition or deletion of nucleotides in homopolymer runs of guanines and cytosines, resulting in frame shifting and loss of its function. Drug-resistant TK mutants retain susceptibility to drugs like foscarnet and cidofovir that are independent of viral-mediated phosphorylation, unless a viral DNA polymerase mutation is also present. Given the essential role of DNA polymerase in viral replication, mutations in this gene occur less frequently and have been found to cluster in functional domains II and III.

5.5.3 Drug-Resistant Varicella-Zoster Virus

Normally, the same drugs are administered for VZV as in the case of HSV. Acyclovir is less effective in this case, while famciclovir and valacyclovir prove to be more competent. Like in the case of HSV, drug resistance in VZV is also attributed to the TK mutations in the viral genome (Lacey et al. 1991). It mainly results due to a premature stop codon that leads to a TK-deficient virus. Other mutations related to resistance appear to cluster at particular VZV TK gene loci. Cross-resistance is seen for drugs acyclovir and penciclovir in some in vitro studies.

5.5.4 Drug-Resistant Cytomegalovirus

CMV is usually an opportunistic pathogen associated with AIDS patients. The principle drugs currently being administered for CMV infections are ganciclovir and valganciclovir. But shortly after the introduction of ganciclovir in the late 1980s, cases of drug resistance came into picture (Akalin et al. 2003). On prolonged exposure to ganciclovir, 90 % of resistant CMV isolates were found to have characteristic mutations in the viral UL97 kinase gene (Chou 2008). These mutations apparently reduce the ganciclovir phosphorylation without impairing the major functions of the kinase in viral replication. CMV UL97 drug resistance mutations cluster tightly at codons 460, 520, and 590–607. Mutations M460V/I, H520Q, C592G, A594V, L595S, and C603W are among the most frequently encountered in ganciclovir-resistant isolates. Apart from this, CMV UL54 DNA polymerase mutations can lead to resistance against almost all the available drugs for CMV infection. Many ganciclovir resistance mutations are located in the exonuclease domains and typically confer cross-resistance to cidofovir whereas mutations in and between the catalytic domains can contribute to foscarnet resistance as well as cross-resistance in cidofovir and ganciclovir in a low grade. Usually, UL97 mutation occurs first with ganciclovir resistance, followed by one or more UL54 mutations after prolonged therapy.

5.5.5 Drug-Resistant Hepatitis B Virus

There are currently seven FDA-approved agents for the treatment of hepatitis B out of which, three are nucleoside analogs (lamivudine, entecavir, and telbivudine) and two are nucleotide analogs (adefovir and tenofovir). All of these target HBV DNA polymerase, which includes reverse transcriptase activity. These kinds of drugs are phosphorylated by cellular enzymes to active form and then incorporated into growing DNA, resulting in premature chain termination, among other inhibitory functions associated with viral replication. Due to the high viral replication rate and the error-prone nature of HBV reverse transcriptase, there has been the emergence of resistance against the above said classes of drugs. Reports suggest that signature mutations in the reverse transcriptase domains of the viral polymerase gene are the main causes of drug resistance as it changes the interaction between the virus and drugs. High-level lamivudine resistance is mostly caused by M204I/V mutations, which are in the YMDD (tyrosine–methionine–aspartate–aspartate) motif in the C domain of the polymerase gene and infrequently by A181V/T mutations. The M204I mutation confers high-level cross-resistance to telbivudine, either alone or in association with the secondary mutations L80I/V or L180M. The N236T mutation, on the other hand, decreases viral replicative capacity in vitro and provides cross-resistance to tenofovir but not to lamivudine or telbivudine. During continued entecavir treatment, additional mutations at I169T and M250V or T184G and S202I are selected, conferring resistance to the same. Report on tenofovir resistance has

been seen in two HBV/HIV co-infected patients with prior lamivudine exposure (L180M/M204V mutations) and an extra A194T mutation (Lacombe et al. 2010).

5.5.6 Drug-Resistant HIV

Resistance of HIV to antiretroviral drugs is one of the most common causes for therapeutic failure in HIV-infected patients. Despite of continuous research and anti-HIV drug development, no combination of drug studied till date has shown to completely block viral replication. Instead, the virus has developed smart mutations in its different survival pathways and continues to be a threat to mankind. Antiretroviral drugs are either nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), or protease inhibitors. Two important mechanisms by which NRTIs work involve mutations (e.g., M184V, K65R, Q151M) occurring at or near the drug-binding site of the reverse transcriptase gene, leading to increased drug discrimination by this gene, and another way is to make key mutations that actually work to undo the action of the drugs, even if they bind to their target RT correctly (Clavel and Hance 2004). NRTIs behave like plugs by blocking nonextendable nucleoside analog monophosphate to the 3′ end of the growing proviral DNA chain, thus inhibiting viral replication. But this phenomenon can be reversed by a reverse transcriptase reaction where the chain-terminating residue is removed and an extendable primer is reinstated. This kind of reverse reaction of DNA polymerization is called pyrophosphorolysis, and it enables reverse transcription and DNA synthesis to resume. This mechanism can be enhanced by some mutations, mostly those selected by zidovudine (Retrovir) and stavudine. As these two drugs are thymidine analogs, these mutations are often referred to as thymidine analog mutations (TAMs).

The mechanism of drug resistance that NNRTIs follow is simpler. All the drugs falling under NNRTIs are designed in such a way to bind to the amino acids packed in a hydrophobic binding pocket within the reverse transcriptase. This pocket does not belong to the active site of the enzyme but near to it and is found only in the presence of the drug. The drugs open this pocket, thus blocking some enzymatic movements inhibiting DNA synthesis. Different mutations conferring resistance to NNRTIs like L100I, Y181C, G190S/A, and M230I involve the amino acids that form the hydrophobic binding pocket. However the K103N mutation is slightly different in the aspect that instead of forming the pocket, it is present near the entrance of the same and it creates a hydrogen bond in the unliganded enzyme. This bond makes the pocket entrance closed for the drug to enter.

Now, the HIV protease gene acts as a homodimer; each of the units constitutes two chains composed of 99 amino acids that make gag (p55) and gag-pol (p160) polyprotein products into active core proteins and viral enzymes (Park and Morrow 1991). These proteases cleave the polyproteins immediately after or during the budding process at nine different positions to give rise to various structural proteins (p17, p24, p7, and p6), reverse transcriptase, integrase, and protease. All the

available protease inhibitors bind to the active site amino acids at the center of the homodimer and prevent cleavage of gag and gag-pol protein precursors in severely infected cells. Hence it arrests the maturation and infection by nascent virions. The virus particles develop resistance by mutations which force the pocket at the center of the homodimer to widen, resulting the drug to freely float and not able to bind to its target tightly.

5.6 *Antiparasitic Drug Resistance*

Parasitic infections have continuously dwindled the global health status, most prominently in the tropical regions. The protozoan and helminth-related diseases have led to the invention of many drugs for their specific treatment decades ago. At that time, about 0.1 % of the global financial asset was invested in therapeutic inventions for tropical diseases including malaria, leishmaniasis, etc. The invention of any drug against targeted pathogens or their structural aspects is an iterative process which involves specific strategies followed by target recognition and validation. The assays after development undergo a thorough screening process to detect structural hits for activity inhibition and their further assessment, and analysis will tag the leads for clinical evaluation. In the case of malaria, drug resistance has been well observed in three malarial species, namely, *P. falciparum*, *P. vivax*, and *P. malariae* (White 2004). With time the parasites have been able to flourish well and replicate within the host system in spite of the drug dosage and absorption. With greater access to parasitic system or the infected erythrocyte, the pharmacokinetics of malarial drugs and understanding of host metabolism have reached new insights. It essentially requires pharmacologically active metabolites for therapeutic purposes. Antiparasitic drug discovery has evolved with new impetus with advancements in genome sequencing, international collaborations, and national programs for fund generation toward this significant impact.

Emergence and Spread The emergence of antiparasitic drug resistance has transformed the global epidemiology profile. Drug dosage and pharmacodynamic properties affect the efficiency of any therapeutic regime. The resistance emergence generally initiates with a genetic change followed by a selection process offering the parasite an endurance profile within host for its survival. Parasitic treatment failures depend on the host immune system as well as parasitic factor. Drug dosage, administration frequency, the period of transition from susceptible to resistant form, time point of host infection, and the fitness cost effects influence the resistance transmission. Acquired tolerance, cross-resistance, drug adherence, and absorption features lead to parasite recrudescence. Cross-resistance is a factor which complicates the administration of different classes of antiparasitic compounds with varied modes of action which are rendered futile due to superbug tolerance mechanisms. The aminoquinolines and antifolates are mostly affected. In malaria, for instance, the non-clearance of asexual parasites leads to gametocyte assembly which

transmits resistance determinants. The resistance development occurs in two phases of tolerance emergence and spread (Klein 2013). Initially a rare, random, and spontaneous genetic mutation provides the parasite a survival advantage which later gets selective, replicates, and becomes insusceptible to treatment. Other than chromosomal mutations, even the gene copies influence the drug target alteration or efflux of therapeutics. A single de novo mutation can lead to multiple events; however, non-immune individuals acting as parasite reservoirs can even contribute to de novo tolerance. Selection of the resistant parasites occurs due to subtherapeutic drug concentration exposure where the sensitive lot succumbs and leads to the drug resistance spread. The time frame for selection and resistance transmission of parasites portrays the "fitness cost" which enables subsequent gametocyte production and cessation of drug activity. The major factors for emergence and spread of antiparasitic drug resistance include the intrinsic frequency of the parasitic genetic mutation, the fitness cost of tolerance, the drug selection pressure, drug pharmacokinetic properties and dosage, host immune profile, and transmission profile. There is a list of extrinsic as well as intrinsic factors that contribute to the emergence of resistance. Access and availability of drugs due to economical hindrances, distribution statistics, and reluctance of usage are the general factors of reduced remedial aid. Additionally, complex antimalarial drug regimes are being self-prescribed by individuals with the threat of inadequate adherence. Unregulated pharmaceutical trade can lead to the commercialization and use of counterfeit antiparasitic drugs. Other than the above mentioned extrinsic factors, the intrinsic elements include parasite's cellular, molecular, and clinical features, their species-based innate resistance, drug activity spectrum, and drug response to parasite's stage susceptibility. All these influence drug resistance in the presence of complete therapeutic adherence. Among parasites, the malarial infections are symptomatic and thereby the individuals develop partial immune response (premunition) against these bugs which keeps a check on the resistance spread. Infection-based immune response selectively eliminates the blood parasites including the de novo resistant strains. The immune status of individuals infected with parasite hereby affects the drug efficiency level. Among the drugs, amphotericin B is the most widely used treatment against leishmaniasis. But the developed resistance is species dependent. The vector control and effective case management hence will enable a check on parasitic diseases.

5.6.1 Genetics of Antimalarial Resistance

With increased resistance, the treatment against the parasites slows down causing increased parasitic recrudescence. The fraction of drug-resistant bugs as compared to sensitive parasites drives the spread of resistance determinants. So with increased resistance, the treatment failures augment accelerating the transmission of resistance. The intracellular parasitic drug concentrations are dependent on the genetic composition of the parasite. The rare spontaneous genetic alterations, additions, or mutations in the genes encoding the drug targets or efflux machinery influence the

parasite tolerance to drug (Wernsdorfer 1991). Changes in the genes may be linked. Mutations regulate the pathogen's fitness disadvantage independent of the drug exposure. Chromosomal mutations are linked to pathogen fitness which reduces with drug exposure. In *P. falciparum*, chloroquine resistance is developed due to alterations in the gene encoding PfCRT and PfMDR1 transporters (Wellems and Plowe 2001). Cytochrome b (*cytB*) single-base deletions cause atovaquone tolerance. Pyrimethamine resistance is due to alterations in dihydrofolate reductase (dhfr) gene (Cowman et al. 1988). Additionally another factor that modulates spontaneous genetic changes is host immunity. Host immunity takes its own time to reach its peak with all weapons to ward off the immune evasion parasitic strategies. The resistance mechanism is specific to the provision of antiparasitic treatment. For instance, most antimalarial treatments are given in response to the asymptomatic features without dipstick confirmation which reduces the chances of resistance selection. Host defense system restricts the parasitic survival by limiting the gametocyte production due to asexual stage as well as antigametocyte immunity. There are even other mechanisms contributing to the parasitic multigenic tolerance. The var gene encodes the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) which undergoes alterations during the course of asexual parasitic cycle. This expresses antigenically variant epitopes for immune responses contributing to distinct surface phenotype. These variant subpopulations don't hamper the transmissible densities. The genomic duplication in *Pfmdr* gene is significantly responsible for contributing to *P. falciparum* resistance to mefloquine (Sidhu et al. 2005). *Pfmdr* is also linked to environmental stress responses and codes *Pgh*, an ATP-dependent P-glycoprotein pump. Antifols tolerance is related to stepwise acquisition of chromosomal mutations in *dhfr* gene. The tolerance for the synergistic recipe of sulfonamides and sulfones with antifols can be attributed due to changes in dihydropteroate synthase gene. *PfATPase6* polymorphism confers artemisinin resistance. Limiting spread of resistance can only put a check on global resurgence of parasitic infections. However, a single point mutation isn't the sole contributor of such events, so deep assessment of *Pfdhfr* gene sequences will cater to very limited advantage. A check on the activation of the gametocytogenesis process can help build up therapeutics. Resistance profile augments the gametocyte carriage which if targeted can curb the resistance spread.

Antimalarial Pharmacokinetics The unbound drug in the host plasma contributes to its therapeutic effect. Innumerable factors like parasitic behavioral, metabolic, and molecular attributes influence the drug effects, and even the drug's pharmacokinetic properties affect the pathogen's response to subtherapeutic treatment levels. The degree of absorption and distribution links the drug's bioavailability within host. The therapeutic ratio and oral bioavailability influences the emergence of resistance. The extended half-life during the drug's elimination phase increases the chances of parasitic encounter with selective drug concentrations. For instance, as chloroquine resistance augments, the drug elimination phase gathers insufficient drug amounts, which decreases the selective nature of tolerance. However, prolonged drug exposure extends the selective capacity of parasites in host

blood system. The transmission again depends on the host immune profile, drug exposure, parasite duplication profile, and host intracellular processes which influence drug-sensitive pathogens. Repeated drug exposure and parasite subpopulation can contribute to drug resistance. The bar of sensitive parasitic MIC if raised will cause a decrease in the susceptible as well as the selective resistant generations. The balance between de novo resistance and drug elimination phase imposes a discriminatory filter for resistance development and susceptibility inhibition. This selection gives an edge for tolerance in parasites highlighting the elimination phase to be instrumental in conferring antiparasitic resistance. The major challenge is the failure in surpassing phase II clinical trials for antiparasitic drugs. The drug synthesis is economically viable and isn't driven by commercial requirements. Yet the detailed insight into the genomics profile for unraveling the translational formulations for novel drug discovery is still a challenge. Secondly, effective partnership for antiparasitics is lacking mainly because they are basically field driven. Proper resources with high-throughput screening for molecular targets that enable optimization of treatment regime will drive the preclinical setups.

The approaches for novel antiparasitic drug discovery will require different entities for innovative therapeutic formulations. Monitoring the therapeutic resistance by investigating into pathogen's phenotypic susceptibility and cellular and molecular alterations in response to drug and developing molecular probes for limiting tolerance can be one of the strategies for refining the present approaches (Pink et al. 2005). The cost and supply of drugs, the diagnostic methodology, and the design of combinatorial therapies can enhance the efficacy of antiparasitic treatments. The combinations are designed in formulations for increased spectrum of activity with either synergistic or additive effect, reduced toxicity, and dosage requirement and prevention of resistance, for instance, combinations like eflornithine and melarsoprol for trypanosomiasis. The combinational therapy works best for combating parasitic infections particularly malaria. Two or more drugs with identical pharmacokinetic properties and different modes of action can slacken the resistance development. Mostly artemisinin derivatives are potent combinatorial medications against malaria due to their pathogen-killing efficiency, reduced toxic effects, and preventive drug resistance features. In combination with mefloquine, artemisinin leaves the mefloquine "tail" unshielded. This drug "tail" eradication phase edges the sieve for resistance emergence. However, the challenges in combination therapeutics that can cause resistance emergence are insufficient treatment and partial population coverage.

Artemisinins are potent drugs for combating malarial infections. The point of remedial action concerns stage specificity. Additionally, parasite doubling and survival have a greater impact and drug targets can inhibit the further disease progression. Antimalarial drug formulations have also employed resistance reverser mechanism. Another approach will involve refining the conventional drugs for broadening the spectrum of drug action. Such indication for therapeutics had been observed in pneumonia medication, DB289, which is now being clinically tested for malaria as well as African trypanosomiasis (Legros et al. 2002). However,

the reluctance of companies to experiment with the risk of testing and toxicity remains an economic challenge. Other strategies involve modulation in drug design for better functioning, like in the case of malarial drugs pyrimethamine whose analogs are being developed to surmount dihydrofolate reductase chromosomal mutations. Antimalarial drug ferroquine bears a chloroquine-like nucleus but with an altered side chain ferrocenic group which lights up its excellent antiparasitic activity against resistant strains (Biot et al. 2005). In vitro tests and molecular approaches ease the parasitic drug monitoring and validation of their therapeutic profile. Chloroquine resistance has been experimented nowadays for producing agents for resistance reversal. Still opportunities for new targets and renewed treatments are being sorted out in the research world today for a ray of anticipation and optimism to combat drug resistance.

6 The Future Ahead

The genesis of drugs/antimicrobial medications gave the world a new hope of survival for fighting the superbugs. Nevertheless, this explosive augmentation of widespread drug usage in the last 20 years has worsened the present global health status by stirring up the tolerance level in microbes. This has also led to the restricted approval of drugs for the present generation of microbial threats. Innumerable complex factors intertwined together contribute to the paucity in the rate of innovative drug development. These multiple forces aren't individually insurmountable, but when combined, their effect imposes a significant and steady proportionate crisis on public welfare with unforeseen consequences. The grave crisis of therapeutic efficacy to combat the microbial resistance mechanisms initiated the introduction of novel strategies and approaches for not only monitoring the drug standards but also limiting the drug tolerance level. This led to the major objective of keeping public health as a priority. Keeping in consideration the past issues, the new generation drugs will employ molecular targets as the line of action against resistance and in vitro tests with molecular markers for drug validation. New drugs specifically new class antibiotics will continue their journey of development for the need of mankind. For instance, medication for multidrug tuberculosis is very much under health requirement. Similarly, *Shigella* outbreaks on a global scale demand cheap antibiotics for oral administration. Generally a huge gap exists between drug development and their clinical approval for worldwide use. This has led to the use of combinational therapies which were found to be effective against many disease outbreaks, malaria being one of them. With a possibility of allergic reactions, these therapies however demanded high-end cost for medications. The problem of generating host-specific "selective pressure" would result in the continued emergence of microbial tolerance. Nevertheless approaches to make sensible usage of drugs along with other essential strategies have decreased the factor of selective pressure. Secondly, early diagnosis might also encourage narrow-range drugs in practice. Two factors are equally important: the duration

of drug therapy and the efficacy of the medication. A keen observation on these parameters would open the doors for the blueprints of next-generation drugs keeping the microbial tolerance level in check. Such surveillance systems also consider the frequency of occurrence, persistence, and spread of drug-resistant organisms to make vital public welfare decisions. Major consideration should focus on the prevention of resistance transmission rather than illness cure when time demands. Vaccine development against fatal diseases and infection control strategies can rather maintain a line of limitation for resistance transmission to humans. The greater incidence of drug resistance puts forward a direct link between clinicians and general public health. The present time demands a greater effective approach to combat resistance for the development of ultra-new class of medications against the superbugs of this era. Otherwise there won't be any delay in the commencement of post-microbial era.

6.1 The Major Hurdles: Challenges

The steady pace of time has enabled the surfacing of fatal infectious pathogens like HIV, human metapneumovirus, etc. Such life-threatening diseases have constrained the pharmaceutical industries for the discovery of safe, novel, effective antiviral drugs with not only increased host life but also limitation in pathogen spread. With such emergence, certain new targets have been defined and unfolded lately. This has resulted in the advancement of medications for AIDS patients either individually or in combinational therapies for increased life expectancy. These life-saving drug discoveries have increased the economical pressure on the R&D sections that have directed their investments for new antiviral drugs at the expense of other general antibiotics. The 5-year report in the USA (1998–2003) shows figuratively similar levels of antibacterial and anti-HIV agents to be discovered and approved by pharmaceuticals and FDA, respectively, during that span of time. Hence, antibacterial research has faced unexpectedly decreased productivity due to greater attrition in the discovery of new antibacterial agents. Even hi-tech throughout techniques and molecular modulations into genome research and computational advancements have failed to stand up to the expectations of defined goals of antibacterial development (Bush et al. 2011). This hindrance elevates the barrier of antibacterial requirement and discovery. The design of present era antibiotics requires certain modified criteria that need to be etched onto the discovery panel for withstanding the present-day challenges of multidrug resistance (Walsh 2003). The same problem continues to persist. Ultimately due to other factors of manufacturing defects, efficacy problem, and economic concerns, there happens to be a halt or delay in the pipeline of antibiotic discovery. The world today requires an urgent transformational change in the field of antibiotic development (Wright 2014). The conventional chemical structures can no longer be experimented with for safe and effective drug breakthroughs. A radical change in invention requires an approach from a different perspective targeting new microbial mechanisms and biological

aspects for a truly novel platform of antibiotic production. An appropriate novel resource intensive strategy will involve greater time and economy as compared to the approach of alteration to the classical drugs. A partnership among the government, academic, and pharmaceutical units can promote the investments for lucrative antibiotic market discoveries. The major limitation is on the restricted antibiotic use for microbial resistance check. These control measures rationalize drug usage thereby lowering their market value and making investments less striking. The challenge of drug development now concerns demand, market profits, as well as technical superiority with desired potency, apt activity, and necessary safe profile for enduring microbial tolerance.

With an expansion of the pathophysiological mechanisms and molecular targets, the spectrum of drug synthesis has increased the opportunities for business turnover. A defined set of "priorities" can enable better investments for refining public lifestyle. Private organizations pool out better drug discovery as compared to the government units, for instance, a 10-year report enlisted a clean sweep of 93 % antibiotic discovery from private organizations as compared to the 3 % from government and academic bodies. The investments are not only a means for meeting the medical needs but also a profitable turnover payoff to stand up for return investment. The pharmaceutical industries invest around 800 million dollars for drug discovery to approval. This figure has significantly increased around fourfold from the nineteenth century. Conversely the increased manufacture cost with prolonged time of 10–12 years of research for drug development (from formulation to clinical approval), patent span of 20 years from the date of invention, and economic burden on pharmaceutical industries have restrained the companies to channelize their priorities for targeted developments. This has narrowed down the spectrum of anti-infective discoveries and their research programs. Overall the combinatorial effects have led to an alarming diminution in antibiotic development. Some incentives can lure private companies for investment start-up in the anti-infective production field. Nevertheless the problem of drug resistance can only be dealt with if more inputs, collaborations, and research are involved in the academic units and research wings for innovative, effective, and safe medications.

6.1.1 Targeting Resistance with Strategic Approach

A deep understanding of pathogen's survival strategy against the drug will provide insights into the molecular targets for discovery of new class of microbial therapy for combating the problem of resistance. For instance, β-lactam resistance facilitated the target alteration of the warhead at the first shot (Mark et al. 2011). With greater clinical menace, β-lactamase-producing strains were targeted for the hydrolase enzyme by mechanistic-oriented inhibition. Augmentin, the combinational therapy of clavulanate and amoxicillin, formed the façade of antibacterial therapy. Another combinational mix with sulbactam and ampicillin marketed under the name of Unasyn was another essential therapy. Sulbactam was an inhibitor of β-lactamase activity similar to other combinational antibacterial treatments like

Timentin and Zosyn. Modulation of tetracycline and erythromycin structures with alteration in their efflux pump strategies would be appropriate antibacterial therapy. Augmentin is under experimentation with certain additive changes of efflux pump inhibition in combination with macrolides or tetracycline. Even certain semisynthetic analogs of vancomycin have been designed for effective activity against vancomycin-resistant strains. The analogs comprise of a vancosamine biphenyl sugar moiety substitution with higher hydrophobicity and more membrane-oriented and greater activity inhibition between transpeptidases and transglycosylases. Even pristinamycin a combination of two synergistic drugs (quinupristin and dalfopristin) inhibits protein synthetic machinery of vancomycin-resistant pathogens.

Another approach to increase microbial drug susceptibility is to disrupt the outer protective barrier that shields the pathogen from the harsh external environment or to target their efflux pump machinery that expels out undesired products thereby preventing drug accumulation. Genetic alteration in *E. coli* (AcrAB) and *Pseudomonas aeruginosa* (Mex efflux machinery) producing efflux knockout strains compromises with the pathogen's resistance mechanism. The synergy among drug resistance mechanisms enables the efflux systems even to affect non-efflux modes of drug tolerance. The inhibitors of MF and RND transporters in *Pseudomonas* and *E. coli* have been well discussed. With such inhibitors, even *gyrA* and *parC* targets against fluoroquinolone resistance are undermined (Cluzet et al. 2015). Such activity of inhibitors even restricted the pathogen tolerance profile of *H. influenza*, *Klebsiella*, etc. The membrane permeabilization mechanism involves cationic peptides as one of the drug-targeted options. A study in *Pseudomonas* reports the cationic peptides having efflux targets along with β-lactamase directed inhibition to highlight interplay of variant modes of microbial resistance (Gellatly and Hancock 2013).

The contribution of multidrug efflux pumps in conferring intrinsic or acquired resistance mechanism isn't validated. In *E. coli*, about seven genes and nine operons have been studied to regulate the intrinsic efflux mechanism, from which very few contribute to antimicrobial resistance. The roles of transporter pump systems like RND, MF, etc., for antimicrobial resistance in Gram-negative bacteria are still under analysis. Many of the functions of these efflux pumps target the release of internal cellular constituents instead of securing the cell from exogenous toxins. Elucidation of substrate recognition mechanism by efflux transporters is still a challenging aspect. Basically the *DNA array* technique for detection of co-regulated genes with efflux systems in induced strains can explicate the functioning of such pumping machinery (Card et al. 2013, 2014). Secondly, certain *genetic alterations* of mutant generation can result in the accumulation of intracellular metabolites, thereby leading to the functional annotation of many transporters. The genomic profile can provide distinct picture for identification of essential multidrug transporter targets that do contribute to intrinsic resistance or can be a factor for acquired resistance mechanism.

The *structural delineation* of many proteins opens gateways to the understanding of molecular mechanism of action. This is true even for elucidation of multidrug

resistance mechanism. The crystal structure of dimeric MarR drug resistance regulator of *E. coli* was solved at 2.3 Å with a DNA-binding domain (Duval et al. 2013). Similar approaches have been employed in deciphering the roles of other transporters that confer resistance mechanism, namely, SmeDEF as well as AcrAB-TolC pump systems. Additionally identification of *tolC* homologue will help to investigate into the mode of antimicrobial resistance in *Vibrio cholerae*.

7 Monitoring Drug Efficacy and Resistance

The four basic methods for monitoring drug efficacy mainly include some in vitro tests, utilization of molecular markers, drug concentration analysis, and detailed drug efficacy studies. Therapeutic effectiveness deals with the direct inspection of drug activity and efficacy over a prolonged period of treatment duration. This clinical standard acts as a blueprint for monitoring subtle alterations consistently for making therapeutic policy outcomes. Following the standard protocol of therapeutic efficacy studies, additional information is also required for the necessary drug characterization and surveillance aspect. Other methods concern the in vitro survival aspect of superbugs and their phenotype depiction, molecular marker experiments regarding genetic knockouts, and drug concentration analysis. The need for a protocol requires a standard test to monitor the in vivo response of the superbugs' resistance causing the disease, for instance, chloroquine resistance against *P. falciparum* (Witkowski et al. 2013). These protocols are modified and revised with the changing drug patterns over time for monitoring the therapeutic proficiency, emergence of resistant strains, and new medications for their treatment. The therapeutic efficacy studies mostly comprise of certain inclusive and exclusive standards, required sample size, assessment parameters, case follow-up strategy, data management and analysis units, ethical committees, and quality check management as the methods of drug evaluation. The treatment is comprised of early medications, clinical failure assessment, and then pathogen response validation. The standard guidelines also involve some national control programs for effective cure policies. However, therapeutic efficacy studies aren't universal methods for monitoring drug remedy parameters. The limitations deal with mostly low sample size of patients for which other surveillance methods and validation techniques (molecular markers/in vitro assessment) can be employed.

7.1 In Vitro Assays

In vitro methods enable to monitor drug efficacy and resistance by evaluating the intrinsic susceptibility of the pathogen to the drugs. The pathogens are given varied drug concentration for generating an optimized and standardized methodology to

gauge their sensitivity profile. These studies can complement the epidemiological breakout of diseases. In vitro studies offer a more broad objective perspective to the problem for determining the resistance mechanism. These tests don't consider host factors to puzzle with the outcome which makes them different from therapeutic efficacy protocols. Most importantly, multiple sample tests can be experimented with a range of drug concentrations against the microbes/parasites. Conversely, the innumerable methods and testing approaches question the comparability as well as compatibility of test outcomes. The various tests include certain radioactive-labeled isotope approach followed by antibody-specific ELISA and certain fluorometric assays. Every step presents a new metabolism profile for data quantification. Different laboratories vary with the same set of experimentations. Data presentation in geometric means scales rather percentage profile can solve the issue of standard protocol requirement. In comparison to therapeutic studies, the outcomes are mostly inconsistent due to lack of a stringent protocol and limitation in monitoring the resistance threshold. In vitro studies have huge complexity with a folly in organization of methodologies. These in vitro tests have a greater advantage to study the combinatorial effects of two or more against the conventional ones (Palomino et al. 2014). A synergistic, antagonistic additive profile can enable useful drug combinations for human welfare. Some factors influencing drug trends can be examined through extended time lengths in such studies. With high technical difficulties and significant variability, the present research encourages the invention of other high-throughput assays for detecting drug resistance and efficacy profile.

7.2 Molecular Markers: Approach for Insights into Drug Resistance

For determination of drug efficacy and understanding of drug resistance mechanism, molecular markers serve one of the most crucial methods. The major genetic alterations responsible for microbial tolerance once identified can be validated and explained with molecular approach. Serving advantageous over other methods, molecular biology experiments can involve greater sample numbers for detection or analysis within restricted time period and the ease of sample storage and transportation as compared to in vitro tests. The genes potentially involved in conferring resistance to drugs after being detected needs to be screened for identification of the molecular markers. Any point mutation at the targeted gene strengthens the pathogen's tolerance level to therapeutics. The greater the frequency and the higher the number of mutational changes, the greater the degree of resistance. For instance, the parasitic dihydrofolate reductase gene is instrumental in contributing to resistance to antimalarial drugs. Sulfadoxine resistance is conferred due to five specific mutations in dihydrofolate reductase gene (Sharma et al. 2015). Higher degree of resistance is contributed due to genetic alterations at three specific positions of 436, 581, and 613, whereas the other positions 437 and

540 add to some amount of parasitic tolerance. The genetic composition of the microbe and the drug response is controlled by many microbial and host factors including drug pharmacokinetic properties. Cumulative genetic modifications forming variant mutants can also influence pathogen clearance and spread of resistance. Increased copy numbers can even attribute to resistance features in pathogens which hampers medication. Several transporter genes encoding putative sodium hydrogen exchange pumps are associated with low drug response. Molecular detection of drug efficacy provides an early indication for geographical monitoring. Such molecular biology methods are essential for detecting the frequency of mutations with drug introduction or withdrawal. Certain drugs bearing technical difficulties of solubility and suspension can be easily tested with such molecular approach. Molecular markers are a direct predictive approach to test the drug efficacy or therapeutic failures that might have led to the selective emergence of resistant species. However, challenges on method sensitivity still continue to persist due to rising mixed pathogen infections in widespread areas. The tests if sensitive will prevent the camouflage of resistant species and avoid discordant outcomes. Due to high consistency in this approach, greater collaborations between research wings and national programmes are being encouraged.

7.3 Drug Concentration Measurement: A Yardstick for Drug Efficacy

The drug design, invention, and its clinical approval take into consideration various physical and absorptive properties of the therapeutic agent before host administration. Thereby it's very essential for understanding the pharmacological and pharmacokinetic properties of any drug before being metabolized and distributed throughout the host system. Intracellular drug absorption, its interaction, host metabolism of drug, and its elimination from the body will influence the dosage of the medication in accordance to its pharmacokinetic properties. The decision of the drug dosage will be required for enabling proper adaptation to the diversified population. For instance, in antimalarial treatment, the poor host absorption property gives rise to variant blood concentrations as the pharmacodynamic features of the drug vary universally. Majorly monitoring the drug efficacy requires consideration of the success rate of the drug or the treatment failures either due to improper dosage or arising microbial resistance strategy. During treatment failure, the drug amount might be slightly less than the MIC of the proliferating superbugs. A lower therapeutic agent dose implies reappearance of the sensitive pathogen after removal of the administered drug with certain drug modulations. Such situations form the argumentative base of microbial resistance mechanism. The drug dosage thereby becomes a very crucial factor for influencing the pharmacological population kinetics of drug variability. Together with such features, certain software modules can formulate these pharmacokinetic attributes and drug characteristic differences

to understand interindividual disparity. For assessing the minimum inhibitory concentrations, research on the drug properties should be intense for further in vivo evaluation. Simple assay method protocols are developed for an approximation of drug exposure. This initiates national control programmes for monitoring the therapeutic agent effectiveness into a routine wide-scale schedule. Additionally, result interpretation would require proper analysis of factors influencing either reduced drug dosage or microbial survival contributing to resistance.

8 Novel Approaches for Drug Development and Resistance Control

The thirst for new drug development with refined microbial targets is under progress. This has led to the development of novel synthetic structures with higher activity range and adequate potency. For instance, the class of oxazolidinones disrupts the protein synthesis by modulating the binding with 23S ribosomal RNA subunit close to the peptidyl transferase junction (Koleva et al. 2015; Jadhavar et al. 2015). Linezolid is an example of clinically approved synthetic oxazolidinones with unmatched potency and selectivity. Another cyclic lipid pentapeptide named ramoplanin equivalent to vancomycin targets substrates of cell wall synthesis. This acts against the vancomycin-resistant *Enterococcus*. The mechanism of drug resistance puts forth two essential queries that require urgent research attention. The first is the approaches that bring up the formulation of new antibiotics and strategies to undermine the resistance development. The first objective necessitates novel approaches for better formulated drugs molecules with efficient delivery and killing mechanism. The second objective requires careful and considerate usage of drug for treating infections.

8.1 Genomics for Anti-Infectives: Quest for Novel Molecular Targets to Circumvent Resistance

The present century now encompasses a huge genomic library database with whole genome sequences of many bacterial pathogens which can further give the researchers a keen insight into the divergent pathogenesis factors (Farhat et al. 2013). Complete sequences of pathogenic strains like *S. aureus*, *Streptococcus* spp., *Salmonella* spp., *M. tuberculosis*, *V. cholerae*, etc., provide a detailed genome profile of the operons regulating the functioning of their antibiotic synthetic mechanism and organization. The gene functional annotation with identical sequences in the database enables identification of targets for mutant library generation. Such functional interruption narrows down the room of choice for targeting the virulence attributes of the pathogen. Such microbial immunomodulatory pathogenic

components enable efficient sustenance within host. These defined pooled down targets responsible for forming the pathogen's survival weaponry once optimized can form a database for automated screening and assessment of inhibitors. The hits of the protein targets can be validated for specificity and efficacy. These hits after validation and optimization can undergo in vitro screening process in cell lines followed up by in vivo mechanistic studies. Such strategies offer significant and secured targets for antibiotic generation. Many examples bring up such bioinformatics analysis (Gautam et al. 2016) followed by wet lab validation for greater success rates of drug development. The famous bacterial metallopeptidase, i.e., peptide deformylase, is the sweet target of many potent drugs which mainly blocks the formyl group transfer (Kumari et al. 2013). The major virulence determinants of microbes that are mostly being screened and targeted for drug development involve the secretion system and the signaling cascade mechanism. The bacterial secretion system forms an injection system that punctures into the membrane barrier for transport of virulence proteins modulating with host functioning. The signaling pathway mainly targets either the two-component network system comprising of a sensor kinase and regulatory transcriptional factor or the quorum sensing mechanism involving differential gene expression in conditional responses. Such diverse spectrum of targets opens up the opportunities for development of novel medications with potential antimicrobial activity.

The library development is categorized into more effective synthetic analogs and natural products. The synthetic groups are large, divergent, and modulated structurally with chemical substitutes and functional groups. Some architectural substitutions are a manner to depict the natural formulations. An in situ library approach of combinational therapy is under research which involves the cloning of the entire stretch of the antibiotic biosynthetic operon clustered within 100 kb of the genome especially for polyketides. This gains advantage over nonculturable microbes. In the interim, the multimodular compilation of the polyketide synthase domain facilitates domain programming, module rearrangement, and swap, substitution, and encoding strategies to formulate a new library of transformed polyketides. The same approach has also been for erythromycin derivative generation. The prospective of such process will require defined and targeted objectives of required mutations, active domain substitutions, and domain swap elements that can form a combinational assembly for new formulations. The libraries can involve many tailoring enzymes involved in the multiple steps of antibiotic biosynthesis be it the additive sugars to the macrocyclic lactone scaffold moiety which are active targets of erythromycin or providing alternate deoxy and amino sugars for in vivo studies.

8.2 New Avenues for Increasing Drug Life Span

The emergence of drug resistance mechanism is competing at par in pace with the development of antibiotics. An inevitable interplay between resistance and

discovery brings in new challenges either to preserve the efficacy of the present therapeutics or to expand the life of such drugs. This explicit approach had to be central to the present era of resistant bacterial havoc. A check has been imposed for considerate drug prescription for the patients as well as for physicians. Inappropriate and unnecessary antibiotic prescription in developed countries can lead to intermittent therapeutic availability, uncertain effectiveness due to regular use, or chances of self-medication leading to worse consequences. Generally subtherapeutic drug dosage can lead to inhabitation of certain bacterial strains at their dormancy state which cause acute complications during medical crisis. At this juncture, the resistance proves dominant over the infection mechanism. So the major issues are with the carriers of dormant infective strains termed as reservoirs. To conserve the efficacy of drugs as the final resort, a rotation principle of antibiotic usage was implied which led to a devastating scenario in the occurrence of vancomycin-resistant MRSA. Not only swap and switch principle but also combinational primary medications have been approved for life-threatening infections where two units work for neutralizing a specific target, but mixture of variant classes target different activities concomitantly. Examples are Augmentin and Synercid specifically. This combinatorial strategy is a standard approach for anticancer regimes and even for antiretroviral therapy to control cancer and AIDS progression. However, the likelihood of emergence of microbial multidrug tolerance increases. Major cases of drug resistance arise during the extended tenure of treatment therapy (McNairy et al. 2013). Previously there have been reports for widespread use of antibiotics as growth promoters in cattle feed. This results in cross-resistance between different host systems which further reduces the antibiotic life span. A study reported the usage of 1000-fold higher vancomycin for human infections when a vancomycin derivative avoparcin was used for animals in the same year (Walsh 2000). When the *Enterococcus* isolates from those animals were screened, similar five operons encoding for vancomycin resistance were found as in the non-infected human carriers. This led to a complete ban of avoparcin. Similar cases have also been reported due to tolerance mechanism of *Enterococcus* against quinupristin/dalfopristin therapy. This resistance was mostly developed due to the use of virginiamycin in Europe in cattle feed since two decades which led to acetyl transferase effect in animal carriers (Walsh 2000). From then, stringency was employed in the approval and usage of antibacterial compounds in cattle feed. The "waste" in feed would cause "haste" in drug development for deteriorating human health. Overall, the modern molecular and high-throughput approaches can enable screening of microbial genes for candidate targets forming the library of novel synthetic and natural molecules that can be experimented and formulated structurally for better functioning. This will crave new genera of modern antibiotics having effective broad-spectrum activity against the conventional ones. The new age antibiotics will however not hamper the resistance cycle, but haphazard use can affect behavioral changes which would be difficult in achieving with regard to antibiotic value.

9 Conclusion

Drug resistance is one of the greatest concerns of modern science. With grave impacts on survival, the potential biochemical and molecular factors for resistance complexity are still under investigation. The research and understanding of pathogen's fitness cost and tolerance dynamics have evolved new opportunities in clinical field for a greater biological interest. The intrinsic procedures make the reversibility process to be sluggish. The quest and urgency for developing new drugs is in pipeline with strategies to circumvent microbial tolerance. New molecular markers, reduced probability of reversibility, and co-selection of resistance mechanism can be used to exploit the fitness cost for choosing drug targets enabling decent predictions on resistance emergence.

9.1 Key Terms and Definitions

1. A **drug** is a natural or synthetic agent which when ingested into the host system stimulates therapeutic effects for disease treatment or prevention.
2. The term **drug resistance** is the reduction in the drug efficacy that defines the ability of microbes to bear or tolerate the drug (chemical or natural agent) dosage that would otherwise inhibit the growth or kill the pathogen.
3. **Intrinsic resistance** defines the inherent/innate property of the microbe to resist the effect of therapeutics due to evolutionary virtue.
4. **Acquired resistance** is the ability the pathogen obtains to withstand the antimicrobial effect due to exogenous gene transfer/exchange methods or majorly due to genetic mutations.
5. **MDR** abbreviates for multiple drug resistance exhibited by the microbe for its insensitivity to a range of antimicrobials.
6. **Antimicrobials** are agents or drugs (natural or synthetic) that modulate the natural functioning of a microbe either by inhibiting the microbial growth or by killing them. Their primary mode of action against microbes designates their classification as antifungals against fungi or antibiotics against bacteria.
7. **Pharmacokinetics** deals with the fate of the drugs after administration within host system. The kinetics of drugs, their absorption, localization, distribution, metabolism, and elimination are the processes studied in pharmacokinetics.
8. **Antibiotics** are medications used to treat bacterial infections, hence termed as antibacterials, for example, penicillins, cephalosporins, etc.
9. **Antimicrobial susceptibility** defines the sensitivity of a particular bacterium or fungus to the dosage of antimicrobial agent thereby affecting the pathogen's growth or survivability.

References

Ahmed M, Lyass L, Markham PN, Taylor SS, Vazquez-Laslop N, Neyfakh AA (1995) Two highly similar multidrug transporters of Bacillus subtilis whose expression is differentially regulated. J Bacteriol 177:3904–3910

Akalin E, Sehgal V, Ames S, Hossain S, Daly L, Barbara M, Bromberg JS (2003) Cytomegalovirus disease in high-risk transplant recipients despite ganciclovir or valganciclovir prophylaxis. Am J Transplant 3:731–735

Alanis AJ (2005) Resistance to antibiotics: are we in the post-antibiotic era? Arch Med Res 36:697–705. doi:10.1016/j.arcmed.2005.06.009

Alekshun MN, Levy SB (1999) The mar regulon: multiple resistance to antibiotics and other toxic chemicals. Trends Microbiol 7:410–413

Alekshun MN, Levy SB (2007) Molecular mechanisms of antibacterial multidrug resistance. Cell 128:1037–1050. doi:10.1016/j.cell.2007.03.004

Almeida Da Silva PE, Palomino JC (2011) Molecular basis and mechanisms of drug resistance in Mycobacterium tuberculosis: classical and new drugs. J Antimicrob Chemother 66:1417–1430. doi:10.1093/jac/dkr173

Alonso A, Sanchez P, Martinez JL (2001) Environmental selection of antibiotic resistance genes. Environ Microbiol 3:1–9. doi:10.1046/j.1462-2920.2001.00161.x

Anderson JB (2005) Evolution of antifungal-drug resistance: mechanisms and pathogen fitness. Nat Rev Microbiol 3:547–556. doi:10.1038/nrmicro1179

Andersson DI, Hughes D (2010) Antibiotic resistance and its cost: is it possible to reverse resistance? Nat Rev Microbiol 8:260–271. doi:10.1038/nrmicro2319

Andriole VT (1999) Current and future antifungal therapy: new targets for antifungal agents. J Antimicrob Chemother 44:151–162

Arnold SR (2007) Revenge of the killer microbe. CMAJ 177:895–896. doi:10.1503/cmaj.071270

Baker-Austin C, Wright MS, Stepanauskas R, McArthur J (2006) Co-selection of antibiotic and metal resistance. Trends Microbiol 14:176–182. doi:10.1016/j.tim.2006.02.006

Balashov SV, Park S, Perlin DS (2006) Assessing resistance to the echinocandin antifungal drug caspofungin in Candida albicans by profiling mutations in FKS1. Antimicrob Agents Chemother 50:2058–2063. doi:10.1128/AAC.01653-05

Begley DJ (2004) Delivery of therapeutic agents to the central nervous system: the problems and the possibilities. Pharmacol Ther 104:29–45. doi:10.1016/j.pharmthera.2004.08.001

Biot C, Taramelli D, Forfar-Bares I, Maciejewski LA, Boyce M, Nowogrocki G, Egan TJ (2005) Insights into the mechanism of action of ferroquine. Relationship between physicochemical properties and antiplasmodial activity. Mol Pharm 2:185–193. doi:10.1021/mp0500061

Bischoff KM, White DG, Hume ME, Poole TL, Nisbet DJ (2005) The chloramphenicol resistance gene cmlA is disseminated on transferable plasmids that confer multiple-drug resistance in swine Escherichia coli. FEMS Microbiol Lett 243:285–291

Bossche HV (1985) Biochemical targets for antifungal azole derivatives: hypothesis on the mode of action. In: McGinnis MR (ed) Current topics in medical mycology. Springer, New York, pp 313–351. doi:10.1007/978-1-4613-9547-8_12

Boswell G, Buell D, Bekersky I (1998) Am Bisome (liposomal amphotericin B): a comparative review. J Clin Pharmacol 38:583–592. doi:10.1002/j.1552-4604.1998.tb04464.x

Brajtburg J, Powderly WG, Kobayashi GS, Medoff G (1990) Amphotericin B: current understanding of mechanisms of action. Antimicrob Agents Chemother 34:183

Brown DF, Reynolds PE (1980) Intrinsic resistance to β-lactam antibiotics in staphylococcus aureus. FEBS Lett 122:275–278

Brumfitt W, Hamilton-Miller J (1989) Methicillin-resistant Staphylococcus aureus. N Engl J Med 320:1188–1196

Bryskier A (2000) Cephems. Fifty years of continuous research. J Antibiot 53:1028–1037

Bush K, Courvalin P, Dantas G, Davies J, Eisenstein B, Huovinen P, Jacoby GA, Kishony R, Kreiswirth BN, Kutter E, Lerner SA, Levy S, Lewis K, Lomovskaya O, Miller JH,

Mobashery S, Piddock LJ, Projan S, Thomas CM, Tomasz A, Tulkens PM, Walsh TR, Watson JD, Witkowski J, Witte W, Wright G, Yeh P, Zgurskaya HI (2011) Tackling antibiotic resistance. Nat Rev Microbiol 9:894–896. doi:10.1038/nrmicro2693

Cannon RD, Lamping E, Holmes AR, Niimi K, Baret PV, Keniya MV, Tanabe K, Niimi M, Goffeau A, Monk BC (2009) Efflux-mediated antifungal drug resistance. Clin Microbiol Rev 22:291–321. doi:10.1128/CMR.00051-08

Capdeville R, Buchdunger E, Zimmermann J, Matter A (2002) Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug. Nat Rev Drug Discov 1:493–502. doi:10.1038/nrd839

Caponigro F, French RC, Kaye SB (1997) Protein kinase C: a worthwhile target for anticancer drugs? Anticancer Drugs 8:26–33

Card R, Zhang J, Das P, Cook C, Woodford N, Anjum MF (2013) Evaluation of an expanded microarray for detecting antibiotic resistance genes in a broad range of gram-negative bacterial pathogens. Antimicrob Agents Chemother 57:458–465. doi:10.1128/AAC.01223-12

Card RM, Warburton PJ, MacLaren N, Mullany P, Allan E, Anjum MF (2014) Application of microarray and functional-based screening methods for the detection of antimicrobial resistance genes in the microbiomes of healthy humans. PLoS One 9, e86428. doi:10.1371/journal.pone.0086428

Chain E (1979) The early years of the penicillin discovery. Trends Pharmacol Sci 1:6–11. doi:10.1016/0165-6147(79)90004-X

Chen X, Ko LJ, Jayaraman L, Prives C (1996) p53 levels, functional domains, and DNA damage determine the extent of the apoptotic response of tumor cells. Genes Dev 10:2438–2451. doi:10.1101/gad.10.19.2438

Chou S (2008) Cytomegalovirus UL97 mutations in the era of ganciclovir and maribavir. Rev Med Virol 18:233–246. doi:10.1002/rmv.574

Clark FS, Parkinson T, Hitchcock CA, Gow NA (1996) Correlation between rhodamine 123 accumulation and azole sensitivity in Candida species: possible role for drug efflux in drug resistance. Antimicrob Agents Chemother 40:419–425

Clavel F, Hance AJ (2004) HIV drug resistance. N Engl J Med 350:1023–1035. doi:10.1056/NEJMra025195

Cluzet VC, Lautenbach E, Nachamkin I, Cary MS, Fishman NO, Shih NN, Morales KH, Linkin DR (2015) Risk factors for gyrA and parC mutations in Pseudomonas aeruginosa. Infect Control Hosp Epidemiol 36:387–393. doi:10.1017/ice.2014.87

Cohen M, Wong E, Falkow S (1982) Common R-plasmids in Staphylococcus aureus and Staphylococcus epidermidis during a nosocomial Staphylococcus aureus outbreak. Antimicrob Agents Chemother 21:210–215

Cohen SP, Hächler H, Levy S (1993) Genetic and functional analysis of the multiple antibiotic resistance (mar) locus in Escherichia coli. J Bacteriol 175:1484–1492

Control, C. f. D, & Prevention (2009) Oseltamivir-resistant 2009 pandemic influenza A (H1N1) virus infection in two summer campers receiving prophylaxis–North Carolina, 2009. MMWR 58:969

Cowman AF, Morry MJ, Biggs BA, Cross GA, Foote SJ (1988) Amino acid changes linked to pyrimethamine resistance in the dihydrofolate reductase-thymidylate synthase gene of Plasmodium falciparum. Proc Natl Acad Sci U S A 85:9109–9113

Cox G, Wright GD (2013) Intrinsic antibiotic resistance: mechanisms, origins, challenges and solutions. Int J Med Microbiol 303:287–292. doi:10.1016/j.ijmm.2013.02.009

Cuenca-Estrella M, Mellado E, Díaz-Guerra TM, Monzón A, Rodríguez-Tudela JL (2000) Susceptibility of fluconazole-resistant clinical isolates of Candida spp. to echinocandin LY303366, itraconazole and amphotericin B. J Antimicrob Chemother 46:475–477

Dahms RA, Johnson EM, Statz CL, Lee JT, Dunn DL, Beilman GJ (1998) Third-generation cephalosporins and vancomycin as risk factors for postoperative vancomycin-resistant enterococcus infection. Arch Surg 133:1343–1346

Davies J, Davies D (2010) Origins and evolution of antibiotic resistance. [Review]. Microbiol Mol Biol Rev 74:417–433. doi:10.1128/MMBR.00016-10

Davis JM, Navolanic PM, Weinstein-Oppenheimer CR, Steelman LS, Hu W, Konopleva M, Blagosklonny MV, McCubrey JA (2003) Raf-1 and Bcl-2 induce distinct and common pathways that contribute to breast cancer drug resistance. Clin Cancer Res 9:1161–1170

De Rossi E, Ainsa JA, Riccardi G (2006) Role of mycobacterial efflux transporters in drug resistance: an unresolved question. FEMS Microbiol Rev 30:36–52. doi:10.1111/j.1574-6976.2005.00002.x

Dean M, Fojo T, Bates S (2005) Tumour stem cells and drug resistance. Nat Rev Cancer 5:275–284. doi:10.1038/nrc1590

Delcour AH (2009) Outer membrane permeability and antibiotic resistance. Biochim Biophys Acta 1794:808–816. doi:10.1016/j.bbapap.2008.11.005

Donadio S, Maffioli S, Monciardini P, Sosio M, Jabes D (2010) Antibiotic discovery in the twenty-first century: current trends and future perspectives. J Antibiot 63:423–430. doi:10.1038/ja.2010.62

Dougherty TJ, Pucci MJ (2011) Antibiotic discovery and development. Springer Science & Business Media, New York

Douglas C, Marrinan J, Li W, Kurtz M (1994) A Saccharomyces cerevisiae mutant with echinocandin-resistant 1, 3-beta-D-glucan synthase. J Bacteriol 176:5686–5696

Duval V, McMurry LM, Foster K, Head JF, Levy SB (2013) Mutational analysis of the multiple-antibiotic resistance regulator MarR reveals a ligand binding pocket at the interface between the dimerization and DNA binding domains. J Bacteriol 195:3341–3351

Dzidic S, Bedeković V (2003) Horizontal gene transfer-emerging multidrug resistance in hospital bacteria. Acta Pharmacol Sin 24:519–526

Englund JA, Champlin RE, Wyde PR, Kantarjian H, Atmar RL, Tarrand J, Yousuf H, Regnery H, Klimov AI, Cox NJ, Whimbey E (1998) Common emergence of amantadine-and rimantadine-resistant influenza A viruses in symptomatic immunocompromised adults. Clin Infect Dis 26:1418–1424

Farhat MR, Shapiro BJ, Kieser KJ, Sultana R, Jacobson KR, Victor TC, Warren RM, Streicher EM, Calver A, Sloutsky A, Kaur D, Posey JE, Plikaytis B, Oggioni MR, Gardy JL, Johnston JC, Rodrigues M, Tang PKC, Kato-Maeda M, Boroesky M, Muddukrishna B, Kreiswirth BN, Kurepina N, Galagan J, Gagneux S, Birren B, Rubin EJ, Lander ES, Sabeti PC, Murray M (2013) Genomic analysis identifies targets of convergent positive selection in drug-resistant Mycobacterium tuberculosis. Nat Genet 45:1183–1189. doi:10.1038/ng.2747

Fischbach MA, Walsh CT (2009) Antibiotics for emerging pathogens. Science 325:1089–1093. doi:10.1126/science.1176667

Fleming A (1944) The discovery of penicillin. Br Med Bull 2:4–5

Flintoff WF (1989) Acquired drug resistance. Genome 31:447

Frost LS, Leplae R, Summers AO, Toussaint A (2005) Mobile genetic elements: the agents of open source evolution. Nat Rev Microbiol 3:722–732. doi:10.1038/nrmicro1235

Gautam A, Chaudhary K, Kumar R, Gupta S, Singh H, Raghava GP (2016) Managing drug resistance in cancer: role of cancer informatics. Methods Mol Biol 1395:299–312. doi:10.1007/978-1-4939-3347-1_17

Gellatly SL, Hancock RE (2013) Pseudomonas aeruginosa: new insights into pathogenesis and host defenses. Pathog Dis 67:159–173. doi:10.1111/2049-632X.12033

Goldrick B (2002) First reported case of VRSA in the United States: an alarming development in microbial resistance. AJN 102:17

Gottesman MM (2002) Mechanisms of cancer drug resistance. Annu Rev Med 53:615–627

Gottesman MM, Fojo T, Bates SE (2002) Multidrug resistance in cancer: role of ATP–dependent transporters. Nat Rev Cancer 2:48–58

Green DW (2002) The bacterial cell wall as a source of antibacterial targets. Expert Opin Ther Targets 6:1–20. doi:10.1517/14728222.6.1.1

Gunn JS (2008) The Salmonella PmrAB regulon: lipopolysaccharide modifications, antimicrobial peptide resistance and more. Trends Microbiol 16:284–290
Hamilton-Miller J (1972) Sterols from polyene-resistant mutants of Candida albicans. J Gen Microbiol 73:201–203
Hartman BJ, Tomasz A (1984) Low-affinity penicillin-binding protein associated with beta-lactam resistance in Staphylococcus aureus. J Bacteriol 158:513–516
Hayes JD, Wolf CR (1990) Molecular mechanisms of drug resistance. Biochem J 272:281–295. doi:10.1042/bj2720281
Heinic GS, Stevens DA, Greenspan D, MacPhail LA, Dodd CL, Stringari S, Struti WM, Hollander H (1993) Fluconazole-resistant Candida in AIDS patients: report of two cases. Oral Surg Oral Med Oral Pathol 76:711–715. doi:10.1016/0030-4220(93)90039-7
Hitchcock C, Whittle P (1993) Chemistry and mode of action of fluconazole. Clin Dermatol 7:183
Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG (2013) Cancer drug resistance: an evolving paradigm. Nat Rev Cancer 13:714–726. doi:10.1038/nrc3599
Jacoby GA (2009) AmpC β-lactamases. Clin Microbiol Rev 22:161–182
Jadhavar PS, Vaja MD, Dhameliya TM, Chakraborti AK (2015) Oxazolidinones as anti-tubercular agents: discovery, development and future perspectives. Curr Med Chem 22:4379–4397. doi:10.2174/0929867323666151106125759
Jones ME, Sahm DF, Martin N, Scheuring S, Heisig P, Thornsberry C, Köhrer K, Schmitz FJ (2000) Prevalence of gyrA, gyrB, parC, and parE mutations in clinical isolates of Streptococcus pneumoniae with decreased susceptibilities to different fluoroquinolones and originating from worldwide surveillance studies during the 1997–1998 respiratory season. Antimicrob Agents Chemother 44(2):462–466
Joseph-home T, Manning NJ, Hollomon D, Kelly SL (1995) Defective sterol Δ5 (6) desaturase as a cause of azole resistance in Ustilago maydis. FEMS Microbiol Lett 127:29–34
Klein E (2013) Antimalarial drug resistance: a review of the biology and strategies to delay emergence and spread. Int J Antimicrob Agents 41:311–317
Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, Harrison LH, Lynfield R, Dumyati G, Townes JM, Craig AS, Zell ER, Fosheim GE, McDougal LK, Carey RB, Fridkin SK, ABCs MRSA Investigators (2007) Invasive methicillin-resistant Staphylococcus aureus infections in the United States. JAMA 298(15):1763–1771
Klopper M, Warren RM, Hayes C, van Pittius NCG, Streicher EM, Müller B (2013) Emergence and spread of extensively and totally drug-resistant tuberculosis, South Africa. Emerg Infect Dis 19:449–455. doi:10.3201/EID1903.120246
Kobayashi N, Nishino K, Yamaguchi A (2001) Novel macrolide-specific ABC-type efflux transporter in Escherichia coli. J Bacteriol 183:5639–5644
Koleva B, Aggen J, Beuning P (2015) Investigation of the mechanism of action of oxazolidinones. FASEB J 29:575.510
Kumari T, Arora R, Kakkar R (2013) Peptide deformylase inhibitors for addressing the issue of bacterial resistance. Int Rev Biophys Chem 4:19–48
Lacey S, Suzutani T, Powell K, Purifoy D, Honess R (1991) Analysis of mutations in the thymidine kinase genes of drug-resistant varicella-zoster virus populations using the polymerase chain reaction. J Gen Virol 72(3):623–630
Lacombe K, Boyd A, Gozlan J, Lavocat F, Girard PM, Zoulim F (2010) Drug-resistant and immune-escape HBV mutants in HIV-infected hosts. Antivir Ther 15:493–497
Lamb DC, Kelly DE, Schunck W-H, Shyadehi AZ, Akhtar M, Lowe DJ, Baldwin BC, Kelly SL (1997) The mutation T315A in Candida albicans sterol 14α-demethylase causes reduced enzyme activity and fluconazole resistance through reduced affinity. J Biol Chem 272:5682–5688. doi:10.1074/jbc.272.9.5682
Lee JY, Ko KS (2012) OprD mutations and inactivation, expression of efflux pumps and AmpC, and metallo-β-lactamases in carbapenem-resistant Pseudomonas aeruginosa isolates from South Korea. Int J Antimicrob Agents 40:168–172

Legros D, Ollivier G, Gastellu-Etchegorry M, Paquet C, Burri C, Jannin J, Büscher P (2002) Treatment of human African trypanosomiasis—present situation and needs for research and development. Lancet Infect Dis 2:437–440. doi:10.1016/S1473-3099(02)00321-3

Lewis K (1994) Multidrug resistance pumps in bacteria: variations on a theme. Trends Biochem Sci 19:119–123

Ligon BL (2004) Penicillin: its discovery and early development. Paper presented at the seminars in pediatric infectious diseases

Lin YM, Wu SJ, Chang TW, Wang CF, Suen CS, Hwang MJ, Chang MD, Chen YT, Liao YD (2010) Outer membrane protein I of Pseudomonas aeruginosa is a target of cationic antimicrobial peptide/protein. J Biol Chem 285:8985–8994. doi:10.1074/jbc.M109.078725

Lina G, Quaglia A, Reverdy ME, Leclercq R, Vandenesch F, Etienne J (1999) Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci. Antimicrob Agents Chemother 43:1062–1066

Little JW, Mount DW (1982) The SOS regulatory system of Escherichia coli. Cell 29:11–22. doi:10.1016/0092-8674(82)90085-X

Löffler J, Einsele H, Hebart H, Schumacher U, Hrastnik C, Daum G (2000) Phospholipid and sterol analysis of plasma membranes of azole-resistant *Candida albicans* strains. FEMS Microbiol Lett 185:59–63

Lomovskaya O, Warren MS, Lee A, Galazzo J, Fronko R, Lee M, Blais J, Cho D, Chamberland S, Renau T, Leger R, Hecker S, Watkins W, Hoshino K, Ishida H, Lee VJ (2001) Identification and characterization of inhibitors of multidrug resistance efflux pumps in Pseudomonas aeruginosa: novel agents for combination therapy. Antimicrob Agents Chemother 45:105–116

Lupetti A, Danesi R, Campa M, Del Tacca M, Kelly S (2002) Molecular basis of resistance to azole antifungals. Trends Mol Med 8:76–81

Maligie MA, Selitrennikoff CP (2005) Cryptococcus neoformans resistance to echinocandins: (1, 3) β-glucan synthase activity is sensitive to echinocandins. Antimicrob Agents Chemother 49:2851–2856

Malouin F, Bryan L (1986) Modification of penicillin-binding proteins as mechanisms of beta-lactam resistance. Antimicrob Agents Chemother 30:1–5

Maravic G (2004) Macrolide resistance based on the Erm-mediated rRNA methylation. Curr Drug Targets Infect Disord 4:193–202

Mariam DH, Mengistu Y, Hoffner SE, Andersson DI (2004) Effect of rpoB mutations conferring rifampin resistance on fitness of Mycobacterium tuberculosis. Antimicrob Agents Chemother 48:1289–1294. doi:10.1128/AAC.48.4.1289-1294.2004

Marichal P, Koymans L, Willemsens S, Bellens D, Verhasselt P, Luyten W, Borgers M, Ramaekers FC, Odds FC, Bossche HV (1999) Contribution of mutations in the cytochrome P450 14α-demethylase (Erg11p, Cyp51p) to azole resistance in Candida albicans. Microbiology 145:2701–2713

Mark BL, Vocadlo DJ, Oliver A (2011) Providing β-lactams a helping hand: targeting the AmpC β-lactamase induction pathway. Future Microbiol 6:1415–1427. doi:10.2217/fmb.11.128

Markham PN, Neyfakh AA (2001) Efflux-mediated drug resistance in Gram-positive bacteria. Curr Opin Microbiol 4:509–514. doi:10.1016/S1369-5274(00)00243-5

Marr KA, Lyons CN, Rustad T, Bowden RA, White TC (1998) Rapid, transient fluconazole resistance in Candida albicans is associated with increased mRNA levels of CDR. Antimicrob Agents Chemother 42:2584–2589

Martel CM, Parker JE, Bader O, Weig M, Gross U, Warrilow AG, Rolley N, Kelly DE, Kelly SL (2010) Identification and characterization of four azole-resistant erg3 mutants of Candida albicans. Antimicrob Agents Chemother 54:4527–4533. doi:10.1128/AAC.00348-10

Martin M, Yates J, Hitchcock C (1997) Comparison of voriconazole (UK-109,496) and itraconazole in prevention and treatment of Aspergillus fumigatus endocarditis in guinea pigs. Antimicrob Agents Chemother 41:13–16

Mazodier P, Davies J (1991) Gene transfer between distantly related bacteria. Annu Rev Genet 25:147–171. doi:10.1146/annurev.ge.25.120191.001051

Mbongo N, Loiseau PM, Billion MA, Robert-Gero M (1998) Mechanism of amphotericin B resistance in Leishmania donovani promastigotes. Antimicrob Agents Chemother 42:352–357. doi:10.1128/AAC.00030-11

McCusker KP, Fujimori DG (2012) The chemistry of peptidyl transferase center-targeted antibiotics: enzymatic resistance and approaches to countering resistance. ACS Chem Biol 7:64–72. doi:10.1021/cb200418f

McNairy ML, Cohen M, El-Sadr WM (2013) Antiretroviral therapy for prevention is a combination strategy. Curr HIV/AIDS Rep 10:52–158. doi:10.1007/s11904-013-0152-1

Molzahn S, Woods R (1972) Polyene resistance and the isolation of sterol mutants in Saccharomyces cerevisiae. Microbiology 72:339–348

Montero C, Mateu G, Rodriguez R, Takiff H (2001) Intrinsic resistance of Mycobacterium smegmatis to fluoroquinolones may be influenced by new pentapeptide protein MfpA. Antimicrob Agents Chemother 45:3387–3392. doi:10.1128/AAC.45.12.3387-3392.2001

Morfin F, Thouvenot D (2003) Herpes simplex virus resistance to antiviral drugs. J Clin Virol 26:29–37. doi:10.1016/S1386-6532(02)00263-9

Müller FMC, Weig M, Peter J, Walsh TJ (2000) Azole cross-resistance to ketoconazole, fluconazole, itraconazole and voriconazole in clinical Candida albicans isolates from HIV-infected children with oropharyngeal candidiasis. J Antimicrob Chemother 46:338–341. doi:10.1093/jac/46.2.338

Nakajima Y (1999) Mechanisms of bacterial resistance to macrolide antibiotics. J Infect Chemother 5:61–74. doi:10.1007/s101560050011

Neu HC (1992) The crisis in antibiotic resistance. Science 257:1064–1073. doi:10.1126/science.257.5073.1064

Nikaido H (1996) Multidrug efflux pumps of gram-negative bacteria. J Bacteriol 178:5853–5859

Nikaido H, Zgurskaya HI (2001) AcrAB and related multidrug efflux pumps of Escherichia coli. J Mol Microbiol Biotechnol 3:215–218

Nugier F, Colin J, Aymard M, Langlois M (1992) Occurrence and characterization of acyclovir-resistant herpes simplex virus isolates: report on a two-year sensitivity screening survey. J Med Virol 36:1–12

Odds FC, Brown AJ, Gow NA (2003) Antifungal agents: mechanisms of action. Trends Microbiol 11:272–279. doi:10.1016/S0966-842X(03)00117-3

Okamura H, Yoshida K, Sasaki E, Morimoto H, Haneji T (2004) Transcription factor NF-Y regulates mdr1 expression through binding to inverted CCAAT sequence in drug-resistant human squamous carcinoma cells. Int J Oncol 25:1031–1038. doi:10.3892/ijo.25.4.1031

Orni-Wasserlauf R, Izkhakov E, Siegman-Igra Y, Bash E, Polacheck I, Giladi M (1999) Fluconazole-resistant Cryptococcus neoformans isolated from an immunocompetent patient without prior exposure to fluconazole. Clin Infect Dis 29:1592–1593. doi:10.1086/313546

Palomino JC, Vandamme P, Martin A (2014) Classical and new assays for detecting drug resistance in tuberculosis. Biomark Med 8:1105–1114. doi:10.2217/bmm.14.73

Park J, Morrow CD (1991) Overexpression of the gag-pol precursor from human immunodeficiency virus type 1 proviral genomes results in efficient proteolytic processing in the absence of virion production. J Virol 65:5111–5117

Park JT, Stromistger J (1957) Mode of action of penicillin. Biochemical basis for the mechanism of action of penicillin and for its selective toxicity. American Association for the Advancement of Science. Science 125:99–101

Paulsen IT (2003) Multidrug efflux pumps and resistance: regulation and evolution. Curr Opin Microbiol 6:446–451. doi:10.1016/j.mib.2003.08.005

Pelletier R, Peter J, Antin C, Gonzalez C, Wood L, Walsh TJ (2000) Emergence of resistance of Candida albicans to clotrimazole in human immunodeficiency virus-infected children: in vitro and clinical correlations. J Clin Microbiol 38:1563–1568

Perea S, López-Ribot JL, Kirkpatrick WR, McAtee RK, Santillán RA, Martínez M, Calabrese D, Sanglard D, Patterson TF (2001) Prevalence of molecular mechanisms of resistance to azole antifungal agents in Candida albicans strains displaying high-level fluconazole resistance

isolated from human immunodeficiency virus-infected patients. Antimicrob Agents Chemother 45:2676–2684. doi:10.1128/AAC.45.10.2676-2684.2001
Petersen EA, Alling DW, Kirkpatrick CH (1980) Treatment of chronic mucocutaneous candidiasis with ketoconazole: a controlled clinical trial. Ann Intern Med 93:791–795
Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Ellis D, Tullio V, Rodloff A, Fu W, Ling TA, Global Antifungal Surveillance Group (2010) Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: a 10.5-year analysis of susceptibilities of Candida species to fluconazole and voriconazole as determined by CLSI standardized disk diffusion. J Clin Microbiol 48:1366–1377. doi:10.1128/JCM.02117-09
Pfaller M, Boyken L, Hollis R, Messer S, Tendolkar S, Diekema D (2005) In vitro susceptibilities of clinical isolates of Candida species, Cryptococcus neoformans, and Aspergillus species to itraconazole: global survey of 9,359 isolates tested by clinical and laboratory standards institute broth microdilution methods. J Clin Microbiol 43:3807–3810. doi:10.1128/JCM.43.8.3807-3810.2005
Piddock LJ (2006a) Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. Clin Microbiol Rev 19:382–402. doi:10.1128/CMR.19.2.382-402.2006
Piddock LJ (2006b) Multidrug-resistance efflux pumps? Not just for resistance. Nat Rev Microbiol 4:629–636. doi:10.1038/nrmicro1464
Piddock LJ, Johnson MM, Simjee S, Pumbwe L (2002) Expression of efflux pump gene pmrA in fluoroquinolone-resistant and-susceptible clinical isolates of Streptococcus pneumoniae. Antimicrob Agents Chemother 46:808–812. doi:10.1128/AAC.46.3.808-812.2002
Piekarska K, Rzeczkowska M, Zacharczuk K, Chróst A, Januszkiewicz A, Bareja E, Olak M, Gierczyński R (2011) Prevalence of qnr genes in clinical Enterobacteriaceae non-susceptible to fluoroquinolone in Poland. Med Dosw Mikrobiol 64:211–219
Pink R, Hudson A, Mouriès MA, Bendig M (2005) Opportunities and challenges in antiparasitic drug discovery. Nat Rev Drug Discov 4:727–740. doi:10.1038/nrd1824
Poole K (2005) Efflux-mediated antimicrobial resistance. J Antimicrob Chemother 56:20–51. doi:10.1093/jac/dki171
Quale J, Bratu S, Gupta J, Landman D (2006) Interplay of efflux system, ampC, and oprD expression in carbapenem resistance of Pseudomonas aeruginosa clinical isolates. Antimicrob Agents Chemother 50:1633–1641. doi:10.1128/AAC.50.5.1633-1641.2006
Rammelkamp CH, Maxon T (1942) Resistance of Staphylococcus aureus to the action of penicillin. Exp Biol Med 51:386–389
Reed JC (2003) Apoptosis-targeted therapies for cancer. Cancer Cell 3:17–22. doi:10.1016/S1535-6108(02)00241-6
Richman DD (2006) Antiviral drug resistance. Antiviral Res 71:117–121. doi:10.1016/j.antiviral.2006.03.004
Ring A, Dowsett M (2004) Mechanisms of tamoxifen resistance. Endocr Relat Cancer 11:643–658. doi:10.1677/erc.1.00776
Ross JI, Eady EA, Cove JH, Cunliffe WJ (1998) 16S rRNA mutation associated with tetracycline resistance in a gram-positive bacterium. Antimicrob Agents Chemother 42:1702–1705
Rubin L, Yolken R, Medeiros A, Moxon ER (1981) Ampicillin treatment failure of apparently β-lactamase-negative Haemophilus influenzae type b meningitis due to novel β-lactamase. Lancet 318:1008–1010
Ryder N (1992) Terbinafine: mode of action and properties of the squalene epoxidase inhibition. Br J Dermatol 126:2–7. doi:10.1111/j.1365-2133.1992.tb00001.x
Sabath L, Laverdiere M, Wheeler N, Blazevic D, Wilkinson B (1977) A new type of penicillin resistance of Staphylococcus aureus. Lancet 309:443–447
Sanglard D, Kuchler K, Ischer F, Pagani J, Monod M, Bille J (1995) Mechanisms of resistance to azole antifungal agents in Candida albicans isolates from AIDS patients involve specific multidrug transporters. Antimicrob Agents Chemother 39:2378–2386
Schmitz FJ, Sadurski R, Kray A, Boos M, Geisel R, Köhrer K, Verhoef J, Fluit AC (2000) Prevalence of macrolide-resistance genes in Staphylococcus aureus and Enterococcus faecium

isolates from 24 European university hospitals. J Antimicrob Chemother 45:891–894. doi:10.1093/jac/45.6.891
Schnappinger D, Hillen W (1996) Tetracyclines: antibiotic action, uptake, and resistance mechanisms. Arch Microbiol 165:359–369
Sekiya-Kawasaki M, Abe M, Saka A, Watanabe D, Kono K, Minemura-Asakawa M, Ishihara S, Watanabe T, Ohya Y (2002) Dissection of upstream regulatory components of the Rho1p effector, 1, 3-β-glucan synthase, in Saccharomyces cerevisiae. Genetics 162:663–676
Sharma J, Khan S, Dutta P, Mahanta J (2015) Molecular determination of antifolate resistance associated point mutations in Plasmodium falciparum dihydrofolate reductase (dhfr) and dihydropteroate synthetase (dhps) genes among the field samples in Arunachal Pradesh. J Vector Borne Dis 52:116–121
Siddik ZH (2003) Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene 22:7265–7279. doi:10.1038/sj.onc.1206933
Sidhu ABS, Valderramos SG, Fidock DA (2005) pfmdr1 mutations contribute to quinine resistance and enhance mefloquine and artemisinin sensitivity in Plasmodium falciparum. Mol Microbiol 57:913–926. doi:10.1111/j.1365-2958.2005.04729.x
Sood S, Malhotra M, Das B, Kapil A (2008) Enterococcal infections & antimicrobial resistance. Indian J Med Res 128:111–121
Stavri M, Piddock LJ, Gibbons S (2007) Bacterial efflux pump inhibitors from natural sources. J Antimicrob Chemother 59:1247–1260. doi:10.1093/jac/dkl460
Strasfeld L, Chou S (2010) Antiviral drug resistance: mechanisms and clinical implications. Infect Dis Clin North Am 24:413–437. doi:10.1016/j.idc.2010.01.001
Sung JML, Lindsay JA (2007) Staphylococcus aureus strains that are hypersusceptible to resistance gene transfer from enterococci. Antimicrob Agents Chemother 51:2189–2191
Tacket CO, Dominguez LB, Fisher HJ, Cohen ML (1985) An outbreak of multiple-drug-resistant Salmonella enteritis from raw milk. JAMA 253:2058–2060. doi:10.1001/jama.1985.03350380074024
Thomas CM, Nielsen KM (2005) Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nat Rev Microbiol 3:711–721. doi:10.1038/nrmicro1234
Tripathi RK, O'Brien RD (1973) Insensitivity of acetylcholinesterase as a factor in resistance of houseflies to the organophosphate Rabon. Pest Biochem Physiol 3:495–498. doi:10.1016/0048-3575(73)90075-8
Vandevelde AG, Mauceri AA, Johnson JE (1972) 5-Fluorocytosine in the treatment of mycotic infections. Ann Inter Med 77:43–50
Veal WL, Nicholas RA, Shafer WM (2002) Overexpression of the MtrC-MtrD-MtrE efflux pump due to an mtrR mutation is required for chromosomally mediated penicillin resistance in Neisseria gonorrhoeae. J Bacteriol 184:5619–5624. doi:10.1128/JB.184.20.5619-5624.2002
Vester B, Douthwaite S (2001) Macrolide resistance conferred by base substitutions in 23S rRNA. Antimicrob Agents Chemother 45:1–12. doi:10.1128/AAC.45.1.1-12.2001
Walsh C (2000) Molecular mechanisms that confer antibacterial drug resistance. Nature 406:775–781. doi:10.1038/35021219
Walsh C (2003) Where will new antibiotics come from? Nat Rev Microbiol 1:65–70. doi:10.1038/nrmicro727
Walsh CT, Fishe SL, Park IS, Prahalad M, Wu PZ (1996) Bacterial resistance to vancomycin: five genes and one missing hydrogen bond tell the story. Chem Biol 3:21–28. doi:10.1016/S1074-5521(96)90079-4
Waxman DJ, Strominger JL (1983) Penicillin-binding proteins and the mechanism of action of beta-lactam antibiotics. Annu Rev Biochem 52:825–869. doi:10.1146/annurev.bi.52.070183.004141
Wellems TE, Plowe CV (2001) Chloroquine-resistant malaria. J Infect Dis 184:770–776. doi:10.1086/322858
Wernsdorfer W (1991) The development and spread of drug-resistant malaria. Parasitol Today 7:297–303. doi:10.1016/0169-4758(91)90262-M

White NJ (2004) Antimalarial drug resistance. J Clin Invest 113:1084–1092. doi:10.1172/jci21682
White TC, Holleman S, Dy F, Mirels LF, Stevens DA (2002) Resistance mechanisms in clinical isolates of Candida albicans. Antimicrob Agents Chemother 46:1704–1713. doi:10.1128/AAC.46.6.1704-1713.2002
Wielders CL, Vriens MR, Brisse S, de Graaf-Miltenburg LA, Troelstra A, Fleer A, Schmitz FJ, Verhoef J, Fluit AC (2001) Evidence for in-vivo transfer of mecA DNA between strains of Staphylococcus aureus. Lancet 357:1674–1675. doi:10.1016/S0140-6736(00)04832-7
Witkowski B, Amaratunga C, Khim N, Sreng S, Chim P, Kim S, Lim P, Mao S, Sopha C, Sam B, Anderson JM, Duong S, Chuor CM, Taylor WR, Suon S, Mercereau-Puijalon O, Fairhurst RM, Menard D (2013) Novel phenotypic assays for the detection of artemisinin-resistant Plasmodium falciparum malaria in Cambodia: in-vitro and ex-vivo drug-response studies. Lancet Infect Dis 13:1043–1049. doi:10.1016/S1473-3099(13)70252-4
Wolfson JS, Hooper DC (1985) The fluoroquinolones: structures, mechanisms of action and resistance, and spectra of activity in vitro. Antimicrob Agents Chemother 28:581–586
Wright GD (2014) Something old, something new: revisiting natural products in antibiotic drug discovery. Can J Microbiol 60:147–154. doi:10.1139/cjm-2014-0063
Wright GD, Poinar H (2012) Antibiotic resistance is ancient: implications for drug discovery. Trends Microbiol 20:157–159. doi:10.1016/j.tim.2012.01.002
Yoneyama H, Katsumata R (2006) Antibiotic resistance in bacteria and its future for novel antibiotic development. Biosci Biotechnol Biochem 70:1060–1075. doi:10.1271/bbb.70.1060