Overview on Mechanisms of Antibacterial Resistance

Alemayehu Toma*, Serawit Deyno

*Pharmacology Unit, School of Medicine, Hawassa University,
Hawassa, Ethiopia

Abstract: Currently antimicrobial resistance among bacteria, viruses, parasites, and other disease-causing organisms is a serious threat to infectious disease management globally. Antibiotics were discovered in the middle of the nineteenth century and brought down the threat of infectious diseases which had devastated the human race. However, soon after the discovery of penicillin in 1940, a number of treatment failures and occurrence of some bacteria such as staphylococci which were no longer sensitive to penicillin started being noticed.

Increasing prevalence of resistance has been reported in many pathogens over the years in different regions of the world including developing countries. This has been attributed to changing microbial characteristics, selective pressures of antimicrobial use, and societal and technological changes that enhance the development and transmission of drug-resistant organisms. Although antimicrobial resistance is a natural biological phenomenon, it often enhanced as a consequence of infectious agents’ adaptation to exposure to antimicrobials used in humans or agriculture and the widespread use of disinfectants at the farm and the household levels. It is now accepted that antimicrobial use is the single most important factor responsible for increased antimicrobial resistance.

The development of antimicrobial resistance by bacteria is inevitable and is considered as a major problem in the treatment of bacterial infections in the hospital and in the community. Despite efforts to develop new therapeutics that interact with new targets, resistance has been reported even to these agents. Searching for new therapeutic possibilities that exist for treatment of bacterial infections and how bacteria become resistant to this therapeutics with combating mechanisms are expected from different stakeholders.

Keywords: antibacterials, mechanisms of resistance, resistance, and microbial

1. INTRODUCTION

WHO’s 2014 report on global surveillance of antimicrobial resistance reveals that antibiotic resistance is no longer a prediction for the future; it is happening right now, across the world, and is putting at risk the ability to treat common infections in the community and hospitals. Treatment failure to the drug of last resort for gonorrhoea – third-generation cephalosporins – has been confirmed in several countries. Untreatable gonococcal infections result in increased rates of illness and complications, such as infertility, adverse pregnancy outcomes and neonatal blindness, and have the potential to reverse the gains made in the control of this sexually transmitted infection. Resistance to one of the most widely used antibacterial drugs for the oral treatment of urinary tract infections caused by E. coli – fluoroquinolones – is very widespread. Resistance to first-line drugs to treat infections caused by Staphylococcus aureus – a common cause of severe infections acquired both in health-care facilities and in the community – is also widespread. Resistance to the treatment of last resort for life-threatening infections caused by common intestinal bacteria – carbapenem antibiotics – has spread to all regions of the world. Key tools to tackle antibiotic resistance such as basic systems to track and monitor the problem reveal considerable gaps. In many countries, they do not even seem to exist (1).

In 2011, there were an estimated 630,000 cases of multidrug-resistant tuberculosis among the world’s 12 million prevalent cases of tuberculosis. Nearly 4% of new cases and about 20% of previously treated cases are multidrug-resistant. Only 50% of multidrug-resistant cases can be effectively treated. On average, the cost for treating one case of multidrug-resistant tuberculosis is equivalent to the cost of treating 100 susceptible tuberculosis cases. An even more severe form of resistance, extensively drug-resistant tuberculosis, has been identified in 84 countries (1).

Beyond the immediate public health impact on morbidity and mortality from these diseases, antimicrobial resistance incurs substantial health-economic and economic costs. The annual cost due
to antibiotic-resistant infections has been estimated to be €1500 million in the European Union and US$ 2000 million in Thailand. The World Economic Forum warned that antimicrobial resistance is one of the major global health security risks that the world needs to tackle and called attention to the fact that losses of gross domestic product from antimicrobial resistance range from 0.4% to 1.6% (2).

Microorganisms have existed on the earth for more than 3.8 billion years and exhibit the greatest genetic and metabolic diversity. They are an essential component of the biosphere and serve an important role in the maintenance and sustainability of ecosystems. It is believed that they compose about 50% of the living biomass. In order to survive, they have evolved mechanisms that enable them to respond to selective pressure exerted by various environments and competitive challenges. The disease-causing microorganisms have particularly been vulnerable to man’s selfishness for survival who has sought to deprive them of their habitat using antimicrobial agents. These microorganisms have responded by developing resistance mechanisms to fight off this offensive (3, 5).

Currently antimicrobial resistance among bacteria, viruses, parasites, and other disease-causing organisms is a serious threat to infectious disease management globally. Antibiotics were discovered in the middle of the nineteenth century and brought down the threat of infectious diseases which had devastated the human race. However, soon after the discovery of penicillin in 1940, a number of treatment failures and occurrence of some bacteria such as staphylococci which were no longer sensitive to penicillin started being noticed. This marked the beginning of the error of antimicrobial resistance. Scientific antibiotic discovery started in the early 1900s by Alexander Fleming, who observed inhibition of growth on his agar plate on which he was growing Staphylococcus spp. It was later found that a microorganism that was later to be called Penicillium notatum was the cause of the inhibition of the Staphylococcus around it as a result of excreting some chemical into the media. That marked the beginning of the discovery of penicillin which together with several other different antimicrobial agents was later to save millions of humans and animals from infectious disease-causing organisms (3, 4).

The observation of Staphylococci spp. that could still grow in the presence of penicillin was the beginning of the era of antimicrobial resistance and the realization that after all the drugs that were described as “magical bullets” were not to last for long due to the selective pressure that was being exerted by the use of these agents. However, the complacency between the 1940s and the 1970s that infectious microorganisms had been dealt a blow was later proved to be a misplaced belief that available antibiotics would always effectively treat all infections. Nevertheless, antimicrobial agents have improved the management of infectious diseases up to date. Increasing prevalence of resistance has been reported in many pathogens over the years in different regions of the world including developing countries. This has been attributed to changing microbial characteristics, selective pressures of antimicrobial use, and societal and technological changes that enhance the development and transmission of drug-resistant organisms (figure 1).

Although antimicrobial resistance is a natural biological phenomenon, it often enhanced as a consequence of infectious agents’ adaptation to exposure to antimicrobials used in humans or agriculture and the widespread use of disinfectants at the farm and the household levels. It is now accepted that antimicrobial use is the single most important factor responsible for increased antimicrobial resistance (1, 4).
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In general, the reasons for increasing resistance levels include the following:

- suboptimal use of antimicrobials for prophylaxis and treatment of infection,
- noncompliance with infection-control practices,
- prolonged hospitalization, increased number and duration of intensive care-unit stays,
- multiple co morbidities in hospitalized patients,
- increased use of invasive devices and catheters,
- ineffective infection-control practices, transfer of colonized patients from hospital to hospital,
- grouping of colonized patients in long-term-care facilities,
- antibiotic use in agriculture and household chores, and
- Increasing national and international travel.

The level of antibiotic resistance is dependent on the following:

- the population of organisms that spontaneously acquire resistance mechanisms as a result of selective pressure either from antibiotic use or otherwise,
- the rate of introduction from the community of those resistant organisms into health care settings, and
- The proportion that is spread from person to person.

All of these factors must be addressed in order to control the spread of antimicrobial-resistant organisms within health care settings. Community acquired antimicrobial resistance is increasing in large part because of the widespread suboptimal use of antibiotics in the outpatient settings and the use of antibiotics in animal husbandry and agriculture (4, 5).

2. MECHANISMS OF ACTION OF ANTIMICROBIAL AGENTS

In order to appreciate the mechanisms of resistance, it is important to understand how antimicrobial agents act. Antimicrobial agents act selectively on vital microbial functions with minimal effects or without affecting host functions. Different antimicrobial agents act in different ways. The understanding of these mechanisms as well as the chemical nature of the antimicrobial agents is crucial in the understanding of the ways how resistance against them develops.
Broadly, antimicrobial agents may be described as either bacteriostatic or bactericidal. Bacteriostatic antimicrobial agents only inhibit the growth or multiplication of the bacteria giving the immune system of the host time to clear them from the system. Complete elimination of the bacteria in this case therefore is dependent on the competence of the immune system. Bactericidal agents kill the bacteria and therefore with or without a competent immune system of the host, the bacteria will be dead. However, the mechanism of action of antimicrobial agents can be categorized further based on the structure of the bacteria or the function that is affected by the agents (figure 2) (6, 9, 10).

These include generally the following:

- Inhibition of the cell wall synthesis
- Inhibition of ribosome function
- Inhibition of nucleic acid synthesis
- Inhibition of foliate metabolism
- Inhibition of cell membrane function

3. MECHANISMS OF ANTIMICROBIAL RESISTANCES

Prior to the 1990s, the problem of antimicrobial resistance was never taken to be such a threat to the management of infectious diseases. But gradually treatment failures were increasingly being seen in health care settings against first-line drugs and second-line drugs or more. Microorganisms were increasingly becoming resistant to ensure their survival against the arsenal of antimicrobial agents to which they were being bombarded. They achieved this through different means but primarily based on the chemical structure of the antimicrobial agent and the mechanisms through which the agents acted. The resistance mechanisms therefore depend on which specific pathways are inhibited by the drugs and the alternative ways available for those pathways that the organisms can modify to get a way around in order to survive (1, 4).

Resistance can be described in two ways:

a) Intrinsic or natural or passive whereby microorganisms naturally do not possess target sites for the drugs and therefore the drug does not affect them or they naturally have low permeability to those agents because of the differences in the chemical nature of the drug and the microbial membrane structures especially for those that require entry into the microbial cell in order to effect their action. An example of natural resistance is Pseudomonas aeruginosa, whose low membrane permeability is likely to be a main reason for its innate resistance to many antimicrobials. Other examples are the presence of genes affording resistance to self-produced antibiotics, the outer membrane of Gram-negative bacteria, absence of an uptake transport system for the antimicrobial or general absence of the target or reaction hit by the antimicrobial (6, 7).

b) Acquired or active resistance, the major mechanism of antimicrobial resistance, is the result of a specific evolutionary pressure to develop a counterattack mechanism against an antimicrobial or class of antimicrobials so that bacterial populations previously sensitive to antimicrobials become resistant. This type of resistance results from changes in the bacterial genome. Resistance in bacteria may be acquired by a mutation and passed vertically by selection to daughter cells (figure 1). More commonly, resistance is acquired by horizontal transfer of resistance genes between strains and species. Exchange of genes is possible by transformation, transduction or conjugation (figure 3) (6, 8). Acquired resistance mechanisms can occur through various ways.

Mechanisms for acquired resistance (7, 8):

- the presence of an enzyme that inactivates the antimicrobial agent
- the presence of an alternative enzyme for the enzyme that is inhibited by the antimicrobial agent
- a mutation in the antimicrobial agent’s target, which reduces the binding of the antimicrobial agent
- post-transcriptional or post-translational modification of the antimicrobial agent’s target, which reduces binding of the antimicrobial agent
- reduced uptake of the antimicrobial agent
- active efflux of the antimicrobial agent
- overproduction of the target of the antimicrobial agent
expression or suppression of a gene in vivo in contrast to the situation invitro
previously unrecognized mechanisms

4. RESISTANCE β-LACTAM ANTIBIOTICS

β-Lactam antibiotics are a group of antibiotics characterized by possession of a β-lactam ring (figure 4) and they include penicillins, cephalosporins, carbapenems, oxapenams, and cephemycins. The penicillins are one of the most commonly used antibiotics in developing countries because of their ready availability and relatively low cost. The β-lactam ring is important for the activity of these antibiotics which results in the inactivation of a set of transpeptidases that catalyze the final cross-linking reactions of peptidoglycan synthesis in bacteria. The effectiveness of these antibiotics relies on their ability to reach the penicillin-binding protein (PBP) intact and their ability to bind to the PBPs (12).

Resistant β-lactams in many bacteria is usually due to the hydrolysis of the antibiotic by β-lactamase or the modification of PBPs or cellular permeability. β-Lactamases constitute a heterogenous group of enzymes which are classified according to different ways including their hydrolytic spectrum, susceptibility to inhibitors, genetic localization (plasmidic or chromosomal), and gene or amino acid protein sequence (11, 12, 13).

5. TETRACYCLINE RESISTANCE

Tetracyclines are another of the very commonly used antimicrobial agents in both human and veterinary medicine in developing countries because of their availability and low cost as well as low toxicity and broad spectrum of activity. The tetracyclines were discovered in the 1940s. They inhibit
protein synthesis by preventing the attachment of aminoacyl-tRNA to the ribosomal acceptor (A) site. They are broad-spectrum agents, exhibiting activity against a wide range of gram-positive and gram-negative bacteria, atypical organisms such as chlamydiae, mycoplasmas, and rickettsiae, and protozoan parasites. Examples of these include drugs such as tetracycline, doxycycline, minocycline, and oxtetracycline (14). Resistance to these agents occurs mainly through three mechanisms, namely

- Efflux of the antibiotics,
- Ribosome protection, and
- Modification of the antibiotic.

These tetracycline resistance determinants are widespread in different microorganisms. Efflux of the drug occurs through an export protein from the major facilitator superfamily (MFS). These export proteins are membrane-associated proteins which are coded for by tet efflux genes and export tetracycline from the cell. Export of tetracycline reduces the intracellular drug concentration and thus protects the ribosomes within the cell. Tetracycline efflux proteins have amino acid and protein structure similarities with other efflux proteins involved in multiple-drug resistance, quaternary ammonium resistance, and chloramphenicol and quinolone resistance. The gram-negative efflux genes are widely distributed and normally associated with large plasmids, most of which are conjugative (15).

Ribosome protection occurs through ribosome protection proteins that protect the ribosomes from the action of tetracyclines. Ribosome protection proteins are cytoplasmic proteins that bind to the ribosome and cause an alteration in ribosomal conformation which prevents tetracycline from binding to the ribosome, without altering or stopping protein synthesis. They confer resistance mainly to doxycycline and minocycline and confer a wider spectrum of resistance to tetracyclines than is seen with bacteria that carry tetracycline efflux proteins.

Modification of the antibiotic on the other hand occurs through enzymatic alteration of the drugs. Some of these genes are coded for by tet(X) genes and otr(X) (14, 15).

6. CHLORAMPHENICOL RESISTANCE

Chloramphenicol binds to the 50S ribosomal subunit and inhibits the peptidyl transferase step in protein synthesis. Resistance to chloramphenicol is generally due to inactivation of the antibiotic by a chloramphenicol acetyltransferase. Various enzymes have been described and are coded for by the cat genes found in gram negative and gram-positive bacteria and usually show little homology. Sometimes decreased outer membrane permeability or active efflux is responsible for the resistance in gram-negative bacteria (16).

7. AMINOGLYCOSIDES RESISTANCE

Aminoglycosides include a group of drugs which are characterized by the presence of an aminocyclitol ring linked to amino sugars in their structure and have a broad spectrum of activity against bacteria. Examples of these drugs include streptomycin, kanamycin, gentamycin, tobramycin, and amikacin, which are commonly used in the treatment of infections by both gram-negative and gram-positive organisms. Their bactericidal activity is attributed to the irreversible binding to the ribosomes but effects resulting from interaction with other cellular structures and metabolic processes are also known (17).

Resistance to aminoglycosides such as gentamicin, tobramycin, amikacin, and streptomycin is widespread, with more than 50 aminoglycoside-modifying enzymes described. Most of these genes are associated with gram-negative bacteria. Depending on their type of modification, these enzymes are classified as aminoglycoside acetyltransferases (AAC), aminoglycoside adenyltransferases (also named aminoglycoside nucleotidyltransferases (ANT), and aminoglycoside phosphotransferases (APH). Aminoglycosides modified at amino groups by AAC enzymes or at hydroxyl groups by ANT or APH enzymes lose their ribosome-binding ability and thus no longer inhibit protein synthesis. Besides aminoglycoside-modifying enzymes, efflux systems and rRNA mutations are involved (17, 18).

8. QUINOLONES RESISTANCE

The first quinolone with antibacterial activity (nalidixic acid) was discovered in 1962 during the process of synthesis and purification of chloroquine. Since then several derivatives have been made
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available on the market, with the most important ones being fluoroquinolones which contain a substitution of a fluorine atom at position 6 of the quinolone molecule. This greatly enhanced their activity against gram-positive and gram-negative bacteria as well as anaerobes. These agents exert their antibacterial effects by inhibition of certain bacterial topoisomerase enzymes, namely DNA gyrase (bacterial topoisomerase II) and topoisomerase IV. These essential bacterial enzymes alter the topology of double-stranded DNA (dsDNA) within the cell. DNA gyrase and topoisomerase IV are heterotetrameric proteins composed of two subunits, designated A and B (19, 22).

Mechanisms of bacterial resistance to quinolones fall into two principal categories:

✓ Alterations in drug target enzymes and
✓ Alterations that limit the permeability of the drug to the target.

The target enzymes are most commonly altered in domains near the enzyme active sites, and in some cases reduced drug-binding affinity. In gram-negative organisms, DNA gyrase seems to be the primary target for all quinolones. In gram-positive organisms, topoisomerase IV or DNA gyrase is the primary target depending on the fluoroquinolones considered. In almost all instances, amino acid substitutions within the quinolone resistance-determining region (QRDR) involve the replacement of a hydroxyl group with a bulky hydrophobic residue. Mutations in gyrA induce changes in the binding-site conformation and/or charge that may be important for quinolone–DNA gyrase interaction. Changes in the cell envelope of gram-negative bacteria, particularly in the outer membrane, have been associated with decreased uptake and increased resistance to fluoroquinolones, and this has not been demonstrated in gram-positive bacteria (20, 21, 22).

9. MACROLIDE, LINCOSAMIDE, AND STREPTOGRAMIN (MLS) RESISTANCE

Resistance MLS antibiotics are chemically distinct inhibitors of bacterial protein synthesis. Intrinsic resistance to MLSB (including streptogramin B) antibiotics in gram negative bacilli is due to low permeability of the outer membrane to these hydrophobic compounds. Three different mechanisms of acquired MLS resistance have been found in gram-positive bacteria (23, 24). These include the following:

- Post-transcriptional modifications of the 23S rRNA by the adenine-N6-methyltransferase which alters a site in 23S rRNA common to the binding of MLSB antibiotics which also confers cross-resistance to MLSB antibiotics (MLS-resistant phenotype) and remains the most frequent mechanism of resistance. In general, genes encoding these methylases have been designated erm (erythromycin ribosome methylation).

- Efflux proteins, which pump these antibiotics out of the cell or the cellular membrane, keeping intracellular concentrations low and ribosomes free from antibiotic, and these have become more frequent in gram-positive populations and often coded for by mef, msr, and vgagenes.

- Hydrolytic enzymes which hydrolyze streptogramin B or modify the antibiotic by adding an acetyl group (acetyltransferases) to streptogramin A have also been described and these confer resistance to structurally related drugs.

10. GLYCOPEPTIDE RESISTANCE

Glycopeptides comprise peptide antibiotics of clinical interest such as vancomycin and teicoplanin. Their antimicrobial activity is due to binding to D-alanyl-D-alanine side chains of peptidoglycan or its precursors, thereby preventing cross-linking of the peptidoglycan chain and thus are largely effective against gram-positive microorganisms which poses a bigger layer of the peptidoglycan although not all gram-positive organisms are susceptible to these agents. High-level resistance to vancomycin is encoded by the vanA gene that results in the production of VanA, a novel D-Ala-D-Ala ligase resulting in the rebuilding of the peptidoglycan side chain to express D-alanyl-D-lactate type which has less affinity for glycopeptides. There are also other proteins in this gene cluster that are necessary for resistance including VanH and VanX, as well as VanB which confers moderate levels of resistance to vancomycin and susceptibility to teicoplanin. Vancomycin gained clinical importance because it was traditionally reserved as a last resort treatment for resistant infections especially of methicillin-resistant Staphylococcus aureus (MRSA). The emergency of vancomycin-resistant organisms has deprived the usefulness of this drug (25, 26).
11. SULFONAMIDES AND TRIMETHOPRIM RESISTANCE

Resistance in sulfonamides is commonly mediated by alternative, drug-resistant forms of dihydropteroate synthase (DHPS). Sulfonamide resistance in gram negative bacilli generally arises from the acquisition of either of the two genes sul1 and sul2, encoding forms of dihydropteroate synthase that are not inhibited by the drug. The sul1 gene is normally found linked to other resistance genes in class 1 integrons, while sul2 is usually located on small non-conjugative plasmids or large transmissible multi-resistance plasmids. Trimethoprim is an analog of dihydrofolate acid, an essential component in the synthesis of amino acid and nucleotides that competitively inhibits the enzyme dihydrofolate reductase (DHFR) (figure 5) (27, 28). Trimethoprim resistance is caused by a number of mechanisms including:

- overproduction of the host DHFR,
- mutations in the structural gene for DHFR, and
- Acquisition of a gene (dfr) encoding a resistant DHFR enzyme which is the most resistant mechanism in clinical isolates.

Fig5. Inhibition of folate synthesis

At least 15 DHFR enzyme types are known based on their properties and sequence homology.

12. CELL MEMBRANE DISRUPTORS: POLYMYXIN ANTIBIOTICS

Cationic cyclic peptides with a fatty acid chain attached to the peptide, such as polymyxins, attack the cytoplasmic membrane of Gram-positive and Gram-negative bacteria and the outer membrane of Gram-negative bacteria. They bind to phospholipids in the cytoplasmic membrane, causing loss of membrane integrity, leakage of cytoplasmic contents and finally cell death. The key initial interaction between the polymyxins and lipopolysaccharides can be blocked by modification of the phosphate esters linked to the diglucosamine components of lipid A (3, 19).

13. MULTIDRUG RESISTANCE

Multidrug resistance among many organisms has become a big challenge to infectious disease management. It is increasingly being reported in bacteria and is often mediated by genetic mobile elements such as plasmids, transposons, and integrons. Integrons are mobile DNA elements with the ability to capture genes, notably those encoding antibiotic resistance, site specific recombination, and they have an integrase gene (int), a nearby recombination site (attI), and a promoter. Integrons seem to have a major role in the spread of multidrug resistance in gram-negative bacteria but integrons in gram-positive bacteria have also been described. Class 1 integrons are often associated with the sulfonamide resistance gene sul1 and are the most common integrons. Class 2 integrons are associated with Tn7. The majority of genes encode antibiotic disinfectant resistance, including resistance to
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aminoglycosides, penicillins, cephalosporins, trimethoprim, tetracycline, erythromycin, and chloramphenicol (18, 23).

Multidrug-resistant strains of Mycobacterium tuberculosis seriously threaten tuberculosis (TB) control and prevention efforts. Molecular studies of the mechanism of action of antitubercular drugs have elucidated the genetic basis of drug resistance in M. tuberculosis. Drug resistance in M. tuberculosis is attributed primarily to the accumulation of mutations in the drug target genes; these mutations lead either to an altered target (e.g., RNA polymerase and catalase-peroxidase in rifampicin and isoniazid resistance, respectively) or to a change in titration of the drug (e.g., InhA in isoniazid resistance) (29).

14. CONCLUDING REMARKS AND RECOMMENDATIONS

The development of antimicrobial resistance by bacteria is inevitable and is considered as a major problem in the treatment of bacterial infections in the hospital and in the community. Despite efforts to develop new therapeutics that interact with new targets, resistance has been reported even to these agents. Searching for new therapeutic possibilities that exist for treatment of bacterial infections and how bacteria become resistant to this therapeutics with combating mechanisms are expected from different stake holders.

REFERENCES


