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Mechanisms of Microbial Resistance to Known Antibiotics

Ahamefule Augustus Kelechi*, Ezeji Ethelbert Uchechukwu

Department of Biotechnology, Federal University of Technology, Owerri, Nigeria

Email address:
kcahams@gmail.com (Ahamefule A. K.), ucheezeji@gmail.com (Ezeji E. U.)

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Abstract: Most infectious diseases are known to be caused by microorganisms. The discovery of antimicrobial agents has saved the human race from a lot of sufferings due to the burden of these infectious diseases. Over the years, microorganisms have developed resistance to known antibiotics. Antimicrobial resistance among bacteria, viruses, parasites, and other disease-causing organisms is a serious threat to infectious disease management globally. Factors responsible for antimicrobial resistance include changing microbial characteristics, selective pressures of antimicrobial use, as well as societal and technological changes that enhance the development and transmission of drug-resistant organisms. Microbial resistance to antibiotics can either be intrinsic or acquired. Different mechanisms of microbial resistance to known antibiotics have been proposed. These include antibiotic inactivation, ribosome protection, biofilm formation, target modification, reduced permeability to antimicrobial agents and increasing efflux of antibiotics from microbial cells. It is believed that the understanding of these mechanisms is important in the discovery of better ways to keep existing agents useful and also in the design of better antimicrobial agents that are not affected by the currently known, predicted, or unknown mechanisms of resistance.

Keywords: Antimicrobial Agents, Diseases, Chemotherapy, Resistance, Microorganisms

1. Introduction

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 [1]. 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 [2]. These microorganisms have responded by developing resistance mechanisms to fight off this offensive. Currently antimicrobial resistance among bacteria, viruses, parasites, and other disease-causing organisms is a serious threat to infectious disease management globally.

Antibiotics, discovered in the middle of the nineteenth century, 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 era of antimicrobial resistance. Scientific antibiotic discovery started in the early 1900s by Alexander Fleming [3], 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 used to save millions of humans and animals from infectious disease-causing organisms [3].

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 [4]. 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 [5].

Increasing prevalence of resistance has been reported in many pathogens over the years in different regions of the world including developing countries [6]. This has been attributed to changing microbial characteristics, selective pressures of antimicrobial use, societal and technological changes that enhance the development and transmission of drug-resistant organisms. Antimicrobial resistance is a natural biological phenomenon. It is however, often enhanced as a consequence of infectious agents' adaptation to exposure to antimicrobials used in humans or agriculture as well as the widespread use of disinfectants at the farm and the household levels [7]. It is now accepted that antimicrobial use is the single most important factor.

2. Mechanisms of Microbial Resistance

Prior to the 1990s, the problem of antimicrobial resistance was never taken to be such a threat to the management of infectious diseases. Gradually treatment failures were increasingly being seen in health care settings against first 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 [8]. 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 in order to survive. Resistance can be described in two ways:

i. Natural/Intrinsic Resistance

Bacteria may be inherently resistant to an antimicrobial agent. This passive resistance is a consequence of general adaptive processes that are not necessarily linked to a given class of antimicrobials. An example of natural resistance is *Pseudomonas aeruginosa*, whose low membrane permeability is likely to be the 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 [9].

ii. Acquired Resistance

Acquired resistance involves bacteria that are usually sensitive to antibiotics, but are liable to develop resistance. Acquired resistance is often caused by mutations in chromosomal genes, or by the acquisition of mobile genetic elements, such as plasmids or transposons, which carry the antibiotic resistance genes. Bacteria are very promiscuous organisms – swapping DNA with each other with ease, even across different species. In such ways, those bacteria which humans have recently been targeting antibiotics at have been able to acquire resistance genes that are already lurking around in the environment [9].

The different mechanisms of antimicrobials resistance are as follows:

2.1. Antibiotic Inactivation

Some bacteria produce modifying enzymes that reside within or near the cell surface, which selectively target and inactivate the drug. Enzymatic inactivation either by hydrolysis or by modification (group transfer and redox mechanisms) is a major mechanism of resistance to natural antibiotics in pathogenic bacteria [10]. The resistant isolates in most cases inherit the antibiotic resistance genes on resistance (R) plasmids. These resistance determinants are most probably acquired by pathogenic bacteria from a pool of resistance genes in other microbial genera, including antibiotic producing organisms. No enzymes that hydrolyse or modify man made antimicrobials have been found. Furthermore, antibiotic inactivation mechanisms share many similarities with well characterized enzymatic reactions and resistance proteins show homologies to known metabolic and signalling enzymes with no antibiotic resistance activity. Therefore, one can speculate that these are the original sources of resistance [11].

Either hydrolysis or group transfer reactions, or alternatively oxidation or reduction reactions, can sign for the inactivation mechanism. Many antibiotics possess hydrolytically susceptible chemical bonds (*e.g.* esters and amides) whose integrity is central to biological activity. When these vulnerable bonds are cleaved, the antibiotic activity is destroyed. The most diverse and largest family of resistance enzymes is the group transferases. Those enzymes covalently modify antibiotics leading to structural alterations that impair target binding. Chemical strategies include *O*-acylation and *N*-acylation, *O*-phosphorylation, *O*-nucleotidylation, *O*-ribosylation, *O*-glycosylation and thiol transfer [12]. The oxidation or reduction of antibiotics has not been frequently exploited by pathogenic bacteria. Lyases are enzymes that cleave C-C, C-O, C-N and C-S bonds by non hydrolytic or non oxidative routes. These reactions frequently result in double bond formation or ring closure.

2.2. Ribosome Protection

Certain bacteria have developed resistance mechanisms that protect the antimicrobial target. For example, in the case of bacterial protein synthesis inhibitors, such as tetracycline, the bacteria have the ability to produce ribosome protection proteins that bind to the ribosomal target thus preventing the binding of tetracycline to the ribosome [13]. Such ribosome protected bacteria will be able to grow in the presence of tetracycline as protein synthesis will be possible. Disease causing bacteria harbouring such ribosome protection mechanisms have been demonstrated to be clinically important.

2.3. Biofilm Formation

A biofilm is an aggregate of interactive bacteria attached to a solid surface or to each other and encased in an exopolysaccharide matrix [14]. This is distinct from planktonic or free-living bacterial growth, in which interactions of the microorganisms do not occur. Biofilms form a slimy coat on solid surfaces and occur throughout nature. A single species of bacteria maybe involved, or more than one species may coaggregate to form a biofilm. Fungi – including yeasts- are occasionally involved.

Biofilms are important in human infections that are persistent and difficult to treat. A few examples include *Staphylococcus epidermidis* and *Staphylococcus aureus* infections of central venous catheters, eye infections such as occur with contact lenses and intraocular lenses, in dental plaque, and with *Pseudomonas aeruginosa* airway infections in cystic fibrosis patients. There are yet many other examples.

The bacteria in the exopolysaccharide matrix may be protected from the host's immune mechanisms. The matrix also presents a diffusion barrier for some antimicrobials, while other antimicrobials may bind to it. Some of the bacteria within the biofilm show marked resistance to antimicrobials in contrast to the same strain of bacteria grown in a free-living broth, which helps to explain why it is so difficult to treat infections associated with biofilms [15].

Biofilm production occurs in many loci, including teeth plaque, water environments, medical catheters, trauma wounds, etc. As such, microorganisms that are found in biofilms are protected from the entry of multiple antimicrobial agents. Thus, biofilms are increasingly becoming a challenge in the human clinical medicine arena [16], when considering potential chemotherapies with antibacterial agents; and this recently recognized new mode of resistance has been reviewed previously.

2.4. Target Modification

Bacteria have found ways to alter the molecular targets of antimicrobial agents. Altered targets may include, for example, DNA gyrase, a target of quinolone antimicrobials, RNA polymerase, a target of rifampin, the prokaryotic ribosome, a target of tetracycline and other protein synthesis inhibitors, and targets of antimetabolite drugs, such as the sulfonamides and related drugs [17]. One classical example of drug target modification is the staphylococcal mechanism of variously altering the penicillin binding protein (PBP) which is the target of β-lactam antibiotics. *Staphylococcus aureus*, the causative agent of serious infectious disease, becomes resistant to these antibiotics by any one of the several mechanisms such as mutation in PBP or acquisition of new PBP with reduced affinity to penicillins, over expression of PBP, etc. Another example of an altered target mechanism includes substitution of amino acids in the quinolone-resistance determining region (QRDR) of DNA gyrase and topoisomerase IV resulting in less efficient binding of quinolone antibiotics [18]. This mechanism has been responsible for widespread quinolone resistance among the Enterobacteriaceae.

Methylation of drug binding targets on 16S rRNA by rRNA methyl transferases is responsible for aminoglycoside resistance in several bacterial species. On the other hand, mutations in genes (*rrs*) encodes ribosomal subunits. The other less studied mechanism of enzymatic degradation is the hydrolysis of the carbon-phosphorus bond in the epoxide antibiotic fosfomycin. This may be enzymatically achieved by a C-P lyase enzyme complex in many Gram negative soil bacteria [19]. The second mechanism of antibiotic inactivation involves enzyme mediated structural alteration of the drug *via* transfer of a functional group such as an acyl, ribosyl, phosphoryl or thiol group. The reaction is irreversible and the modified antibiotic is unable to bind to the target due to the resultant change in the structure. The antibiotics susceptible to this bacterial mechanism include aminoglycosides, fosfomycin, macrolides, lincomycin and chloramphenicol [20]. For instance, bacteria have evolved acetyl transferases which inactivate chloramphenicol, tetracycline-metabolizing enzymes that are largely uncharacterized, and beta-lactamases that inactivate beta-lactams such as penicillin. Highly active variants of these enzyme inactivation mechanisms for drugs are ubiquitous in the environment and have yet to be found within clinically relevant bacterial pathogens [21]. The enzymatic *O*-acetylation of chloramphenicol by chloramphenicol acetyltransferase (CATs) is responsible for the inactivation of this drug. Similarly, the modification of aminoglycoside antibiotics into their inactive forms leading to bacterial resistance is achieved by aminoglycoside acetyltransferases or AACs. The enzymes of this group vary in their choice of groups (hydroxyl or amino) as well as their positions on aminoglycoside antibiotics for acetyl group transfer, but their actions invariably lead to drastically reduced affinity of the antibiotics to their ribosomal targets [22]. The other enzyme-mediated inactivation of antibiotics include acetylation of streptogramins by streptogramin acetyl transferases (VATs, for virginiamycin acetyl transferases), aminoglycoside modification by aminoglycoside phosphotransferases (APHs), phosphorylation of macrolides by macrolide kinases (MPHs), glutathione induced fosfomycin inactivation by FosA (or FosB), ADP-ribosylation of rifampin by ADP-ribosyltransferases (ARRs), nucleotidylation of aminoglycosides and lincomycin by nucleotidyl transferases (ANTs and Lin), glycosylation of macrolide antibiotics by glycosyltransferases. A less common mechanism is the inactivation of an antibiotic by redox process which involves flavin dependent monoxygenase enzyme TetX. This enzyme transfers a single hydroxyl group to tetracycline at position 11a resulting in a structure that is less able to sequester Mg+ ions which are critical for binding of tetracycline to its bacterial target [23]. TetX is present on a transposon, and this mechanism has been recently found to be responsible for bacterial resistance to a third generation tetracycline, tigecycline.

2.5. Reduced Permeability

A drug resistant phenotype of a bacterium may arise due to the inability of the antimicrobial agent to gain entry into the cell where the drug targets are located. One mechanism that results in reduced drug permeability in bacteria is the cell wall's lipopolysaccharide (LPS), which consists of lipid A, a core consisting of polysaccharide and O-antigen . Bacteria that harbour LPS moieties show resistance to erythromycin, roxithromycin, clarithromycin and azithromycin in Gram-negative bacteria such as strains of *Pseudomonas aeruginosa*, *V. cholerae* and *S. enterica*, all of which are serious pathogens, especially in immune-compromised patients [24]. Another mechanism that confers reduced permeability involves the porin channels that reside in the outer membrane and allow small molecular weight molecules, such as antimicrobial agents, to gain cellular entry. Drug resistant bacteria alter the expression of these outer membrane proteins such that they fail to integrate into the outer membrane or are functionally defective, thus preventing the entrance of growth-inhibitory molecules [2]. Clinically important bacterial pathogens like *Serratia marcescens, E. cloacae, S. enterica, E. aerogenes, Klebsiella pneumoniae*, and *P. aeruginosa*, have utilized this reduced drug uptake system to resist important antimicrobial agents, such as the betalactams, fluoroquinolones, aminoglycosides, as well as chloramphenicol [25].

2.6. Efflux Pumps

Increasing the efflux also plays a role, especially with hydrophobic compounds that presumably enter the cell *via* diffusion. At the same speed where these antimicrobials are entering the cell, efflux mechanisms are pumping them out again, before they reach their target. A mutation resulting in overexpression of a multidrug efflux pump leads to resistance to a wide variety of structurally unrelated antimicrobials [25]. Multidrug resistance proteins (MDRs) or multidrug efflux pumps are widespread in bacteria. They are grouped into five families based on their mechanisms and primary sequence homologies. The major facilitator super (MFS) family, the resistance-nodulation-division (RND) family, the small multidrug resistance (SMR) family and the multidrug and toxic compounds extrusion (MATE) family are secondary transporters using either proton motive force (PMF) or sodium ion motive force (only for the MATE proteins) to expel antimicrobials from cells. Members of the ATP binding cassette (ABC) superfamily are primary transporters using energy liberated by ATP hydrolysis.

3. Conclusion

We conclude this review with an excerpt from the Nobel Prize laureate, Joshua Lederberg: "*Antibiotic resistance as a phenomenon is, in itself, not surprising. Nor is it new. It is, however, newly worrying, because it is accumulating and accelerating, while the world's tools for combating it decrease in power and number*."

This description may sound gloomy, but unfortunately, it is rather precise. We must remember that microbes have been on earth much longer than man and can develop resistance to any antibiotics used to treat them. It is therefore recommended to use a combination of approaches to minimize the resistance problem, and hopefully man can live in peace with the microbes.

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