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Minireview Article

The basic of bacterial resistance to antimicrobial drugs

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ABSTRACT

Drug-resistant organisms may have acquired resistance to first-line antibiotics, thereby obligating the use of second-line agents and so this problem is a serious and pre-eminent public health concern in the 21st century. Evolutionary pressure caused by misuse and overuse of antibiotics has played a role in the development of multidrug-resistant varieties and the spread of resistance among bacterial species. Some pathogens, such as Pseudomonas aeruginosa, also dominate a high level of intrinsic resistance. This review discusses biochemical and genetic ways that bacteria use to become resistance against antimicrobial agents and authors interested to show the emergence of this global problem. Some new approaches to fight against our microscopic enemies should be discussed in this article.

***Keywords*:** Antibiotic resistance, resistant pathogens, resistant genes, horizontal gene transfer

INTRODUCTION

Alexander Fleming warned against the use of sub-therapeutic doses of antibiotics – "bought by anyone in the shops" without a prescription [1]. In 1945, when Fleming alarmed about antibiotics use in his Nobel lecture "Penicillin," knowledge about antibiotic resistance was very little but the number of published articles subjected antibiotics is extremely increased in last

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decade, today we could say alarm bells are ringing for antibiotic resistance [2,3].

There are some ways for bacteria to be resistant such as spontaneous or induced genetic mutation, or the acquisition of resistant genes from other bacterial species by horizontal gene transfer via conjugation, transduction, or transformation [4,5]. The use of antibiotics in animals is also partly responsible for the emergence of antibiotic-resistant microorganisms in human medicine. Also, there are resistant bacteria that live in animal bodies that could be transmitted to human by three pathways, the consumption of animal products (milk, meat, eggs), direct contact with animals or other humans and through the environment [6,7]. Systematic and meta-analysis shows that limiting the use of antibiotics in food-producing animals is associated with the reduction of antibiotic-resistant bacteria in these animals. [8]. Therefore the world needs for coordinated action to reduce the spread of antimicrobial resistance. A gene for antibiotic resistance spread through an ecosystem of bacteria by antibiotic-resistance plasmids which contain genes conferring resistance to several different antibiotics [9].

Although there were low levels of preexisting antibiotic-resistant bacteria before the widespread use of antibiotic, there is some evidence of resistant strains existence before treating by penicillium in 1952 or resistance of clostridium to clindamycin cause by a random mutation in the genome. Also, heavy metals and pollution play a role in antibiotic resistance in the bacterial niche [10].

So there is a natural occurrence of antibiotic resistance without misusing or even correct using of antibiotics. Some genes that confirm this resistance are known as the environmental resistome. They may be transferred from non-disease-causing bacteria or other resistomes such as animals to those that do cause disease, leading to significant antibiotic resistance [11]. In this paper, we reviewed the mechanisms of drug resistance in bacteria with an emphasis on genetic and biochemical aspects. This paper reviewed the mechanisms of drug resistance in bacteria, with emphasis on genetic and biochemical aspects, and introduces some resistant species and origin of antimicrobial resistance. Also, some new ways to cope with drug resistance are discussed.

Resistant pathogens

The major resistant pathogens included Streptococcus and Enterococcus, Staphylococcus, Pseudomonas aeruginosa, Clostridium difficile, Salmonella and E. coli, Acinetobacterbaumannii, Klebsiella

pneumonia, Mycobacterium tuberculosis and Neisseria gonorrhoeae [12].

For example, Staphylococcus aureus, found on mucous membranes and on the skin about one-third of the world's population, is highly consistent with antibiotic treatment. Just four years after the use of penicillin as an antibiotic, this bacterium becomes resistant. And Methicillin-resistant Staphylococcus aureus (MRSA) was first detected in Britain [13]. However, S. aureus infections in the United States are resistant to penicillin, methicillin, tetracycline, and erythromycin.

M. tuberculosis (TB) is another example of the resistant bacteria which develops resistance to drugs by spontaneous mutations in its genomes. 150,000 deaths annually are caused by Multidrug Resistant TB (MDR TB) in the whole world [14].

Neisseria gonorrhea has a high affinity for horizontal gene transfer, so resistant strains develop easily. Sometimes a combination of injectable ceftriaxone with azithromycin or doxycycline is used to treat this infection [15].

Origins of antimicrobial resistance

Resistant genes and mechanisms of transfer probably existed before the use of the modern therapeutic antimicrobial agent [10].For instance, scientists identified E. coli resistant to sulfadiazine, spectinomycin, and tetracycline earlier than 1950. These findings emphasize that resistance is not a new phenomenon and it may arise within antibiotic-producing microorganisms as a mechanism to protect them against auto-toxicity. This popular belief supported by evidence that upholds the finding of aminoglycoside-modifying enzymes in aminoglycoside-producing organisms similar to modifying enzymes was found in aminoglycoside resistant bacteria [16].

Mechanisms of resistance

Biochemical mechanisms

Resistant Bacteria may have either intrinsic resistance or acquired resistance. Intrinsic resistance is a natural phenomenon displayed by all members of a species as a physiological or biochemical function [17]. For example, enterococci are resistant to cephalosporins intrinsically as a result of a decreased binding affinity to the penicillin-binding proteins. On the other side acquired resistance can result from mutations in the genome, acquisition of resistance genes or a combination of these two mechanisms. Acquired resistance is only present within a certain lineage of bacteria not in the entire species [4].

In contrast to different methods of acquiring resistance to bacterial species, resistance is created by a number of mechanisms, such as

antibiotic inactivation, target modification, efflux pumps, and outer membrane (OM) permeability changes, and bypassing the target. The biochemical aspects of antibiotic resistance are shown in figure 1.

Antibiotic resistance mechanisms are diverse in each of these four categories and a bacterial strainuses various ways against antibiotics. The choice of mechanisms depends on the nature of the antibiotic, its target site and the source of resistance, whether it is due to resistance plasmid or by chromosomal mutation. For example, biochemical strategies for antibiotic inactivation are hydrolysis, group transfer, and redox mechanisms mediated by enzymes [18]. Some resistant bacteria escape from antimicrobials by changing or covering target sites to avoid recognition. Consequently, despite the presence of antibiotics in the cell, these substances cannot be bound to their target sites. Bacteria use the following strategies to modify target sites: alteration in penicillin-binding protein (PBPs), changes in peptidoglycan layer and cell wall thickness, alterations in subunits of DNA gyrase, topoisomerase, and RNA polymerase [19]. Efflux pumps act synergistically with OM permeability to reduce the concentration and accumulation of the antibiotics in the bacterial cells. Although the transfer of proteins and enzymes is a specific process, some of the effluxpumps are multidrug and recognize a broad range of different substrates and antibiotics [20,21].

Genetic mechanisms

In brief, we can divide genetic aspects of antibiotic resistance into two zones including mutations and gene transfer.

For a long time, mutations has been thought that resistance occurrence in microorganism needs a large population. Nowadays, we know that there is no necessity for large populations. For example, small populations of E.coli can acquire resistance in an antibiotic gradient which may facilitate the development of antibiotic resistance because of arising four SNP mutations in the genome of *E.coli* [22].

Antibiotic resistance can be due to horizontal gene transfer as well as point mutations in the pathogen genome at a rate of about 1 in 108 for each chromosomal replication. Those bacteria with a mutation that allows them to survive against antibiotics and then they can pass this profit to their offspring. So we can see that the antibiotics act as an environmental pressure which leads to the evolution of resistant colony [23]. It seems that gain and maintenance of resistance genes in a bacterial population is caused by selective antibiotic pressure [24].

Resistance gene has been found for most antibiotic classes and gene products are involved in various mechanisms of resistance such as such efflux, target bypass, and drug inactivation.

Resistance based on point mutations often alters the binding targets of antibiotics, resulting in a decreased binding affinity. For example, point mutations in the gene encoding DNA gyrase decreased binding affinity of quinolones, or multiple point mutations of DNA topoisomerase genes, such as gyrA, gyrB, and parC, decreased susceptibility to quinolone antimicrobials [25].

Another example of mutation causing resistance is about macrolides which bind to the ribosome and interfere with protein synthesis. Genomic mutations of ribosomal 50S subunit change the binding site and can decrease the efficiency of macrolide binding and result in resistance to macrolides[17].

Decreases in bacterial cell permeability are the other reason for antibiotic resistance which can be caused by mutation. Outer membrane proteins (Omps) are determinants of entry for molecules to the cell membrane, including antibiotics. Mutation of these porins can cause resistance to a wide variety of antibiotics. Mutations in the outer membrane lipopolysaccharides (LPS) genes can contribute to resistance as well [26].

Active efflux is a mechanism employed by bacteria to decrease the concentration of antibiotics. Resistance mediated by Efflux is usually attributed to mutations in genes of the efflux system via an increase in the expression of the efflux pump protein or in amino acid substitutions that makes the efflux pump more efficient in exporting antimicrobials out of the cell [27, 28].

Also, some biochemical mechanisms of antibiotic resistance depending on mutation, like the mutations of genes encoding the target of certain antibiotics such as mutations in RpoB and DNA-topoisomerases make the microorganism resistance to rifamycins and fluoroquinolones, respectively [29].

We explained the mutations which lead to resistance by spontaneous mutation occurring randomly as replication errors or an incorrect repair of a damaged DNA. Despite the mechanisms of the cell to maintain the stability of genetic information, the existence of bacteria with an elevated mutation rate proofed. Hypermutablestrains or mutators have a high frequency of mutations and play an important role in the evolution of antibiotic resistance. Hypermutators have been found in populations of E. coli, Salmonella enterica,

Neisseria meningitidis, Haemophilus influenzae, Staphylococcus aureus, Helicobacter pylori, Streptococcus pneumoniae, and P. aeruginosa [30].

Under certain conditions (e.g. non-lethal antibiotic selective pressure) mutators may accelerate the evolution of favorable mutations such as resistance and these mutators can be fixed in the population during this process. The achievement of a useful mutation, allow the mutators to relieve the selective pressure and the cells begin to grow [31].

Another genetic aspect of acquired antibiotic resistance is gene transfer which is divided into two basic ways, vertical transmission and horizontal transmission, schematically shown in figure 2.

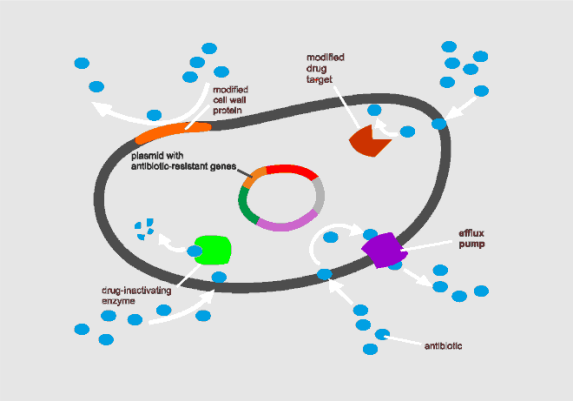

Figure 1. Four mechanisms of resistance: (**1**) Impermeable barrier blocks antibiotics entrance to the cell. (**2**) Target modification alters the sensitivity of proteins inhibited by the antibiotic (**3**) Antibiotic inactivation by an enzyme. (**4**) Efflux pump reduces the concentration of drug by employs enzymes that actively pump the antibiotic out of the cell. (Blue spheres are antibiotics) [10].

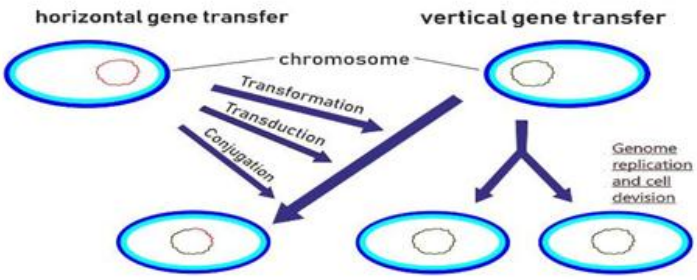

Figure 2. Horizontal and vertical gene transfer [11].

Table 1. Some examples of resistance transposons in gram-positive bacteria

Transposon *	Antibiotic resistance
Tn 551	Erythromycin
Tn 917	Erythromycin
Tn 4451	Chloramphenicol
Tn 4001	Gentamicin, Kanamycin, Tobramycin
Tn 4003	Trimethoprim

* Transposons appear in different forms and determinated by structure, genetic and transport mechanism

Table 2. Some examples of resistance transposons in gram-negative bacteria

Transposon	Antibiotic resistance
Tn 1, Tn 3	Ampicilin
Tn 21	Mercuric ions, Streptomycin, Sulphonamide
Tn 501, Tn 3926	Mercuric ions
Tn 1721	Tetracycline
Tn 5	Bleomycin, Kanamycin, Streptomycin
Tn 9	Chloramphenicol
Tn 10	Tetracycline
Tn 903, Tn 1525, Tn 2350	Kanamycin

* Transposons appear in different forms and determinated by structure, genetic and transport mechanism

The example of horizontal gene transfer is tetracycline resistance which is due to the acquisition of new genes often associated with mobile elements. These genes are usually associated with plasmids or transposons and are often conjugative. Particular plasmids, transposons, andintegrons can exchange the resistance in bacteria. Plasmids which carry the resistance genes are called R plasmids. Many resistance elements are transmitted by transposons via insertion into a conjugative plasmid or a conjugative transposon. Conjugative transposons appeared to be a hybrid between transposons and plasmids. Over the past decades, the assumption was that conjugative transposon were found mainly on gram-positive bacteria [32], but later it was observed that some transposons easily relate to a wide range of gram-negative bacteria[33,34]. What is illustrated (Tables 1, 2) are just a few of known transposons [35,36]. Conjugation occurs when genes are transferred between bacterial cells through tubes called pilli [37].

Gene cassettes are free circular DNA structures with antimicrobial resistance genes which cannot be expressed alone because of

the lack of the promoter region. These gene cassettes can be inserted into integrons in recombination site. Now, these integrons can transfer the resistance gene. A novel genetic system for the movement of antibiotic resistance genes is integron. Integrons are located either on the bacterial chromosome or plasmids. Integronsare genetic units characterized by their ability to capture and incorporate gene cassettes by site-specific recombination [38-40].

Mobile DNA elements such as plasmids, transposons, and integrons could transport multiple antimicrobial resistance genes and are responsible for dissemination of these genes between bacterial species. Also, antimicrobial resistance determinants can be spread among bacteria via transformation and transduction.

Transformation arises when bacteria acquire resistance genes from dead bacterial cells and integrate them into their own genomes. Transduction is a process in which resistance genes are transferred from one bacterium to another via bacteriophages.

Horizontal transfer of resistance genes is a mechanism for spreading multiple drug resistance (MDR) because resistance genes can be transferred together. Integrons with the ability to capture genes, such as antibiotic-resistant genes, are mobile DNA elements which cause MDR [41].

CONCLUSION

Widespread uses of antibiotics cause the epidemics of antimicrobial resistance around the world. Resistances in some species develope so that no clinically available treatment is effective. The British society for antimicrobial chemotherapy (BSAC) meeting 2014 warns if the number of hard-to-treat infections continues to grow, it will become difficult to control infection in humans. World economic forum (WEF) reports 2014 highlights antibiotic resistance. However, only two classes of new antibiotics have been brought to the market in the last 30 years so it seems that the development of new antibiotics is essential. In this regard Upsala university gets to lead a large research cooperation project for new antibiotics, ”ENABLE”, financed with € 85 million over six years. The European gram-negative antibacterial engine (ENABLE) project spans 13 countries to establish a significant anti-bacterial drug discovery platform with the goal to deliver at least one new anti-bacterial candidate against gram-negative bacteria up to 2019. Certainly, we need novel approaches and new ways to fight back against antibiotic resistance. One idea is to “develop viruses to

fight resistant bacteria", the headline of the New York Times in 2013 December. This is based on the idea that disease-causing bacteria have their own lethal viruses. In contrast to chemical antibiotics, viruses evolve and so bacteria are unable to become resistant to all of their viruses. Matti Jalasvuori said "The enemies of our enemies can be used to cause severe epidemics among problematic bacteria" and published articles whit this subject [42,43]. Engineered bacteriophage can be used to attack gene networks of bacteria and enhance the killing of antibiotic-resistant bacteria [44]. Nanoparticle-based treatments are other approaches as highly effective bactericidal material. These nanostructures should be able to store the drugs in bacterial cells and thereby resolve the resistance problem [45,46].

Some researchers utilize the bacterial traits to design antibacterial components such as riboswitch-based therapeutics. Riboswitches control genes are essential for bacterial survival and bacterial infection, because of these abilities scientists optimize to formulate a drug to affect a riboswitch and therefore shutting down pathogenic bacteria [47]. Another example is two-component systems as potential targets for antimicrobial therapy because they are widespread in bacteria and, so far, absent in mammals. Histidine kinases (HK) and response regulators (RR) are two components that allow bacteria to sense and response the external signals, therefore, general HK or RR inhibitors could potentially be broad-spectrum antibiotics that act by slowing down the intracellular networks which cause cellular shutdown [48,49]. It appears that we are in a game with bacteria looking for new ways to keep pathogens in check, also searching for even newer antipathogen strategies and pathogens tap into the neighbor's resistomes and acquired resistance genes. Interconnections between people, animals, and the environment make it easy for antibiotic-resistant bacteria to transfer their genes from one resistome to another. For instance, a resistant strain which lives in the soil could travel to humans via drinking water or swimming [11].

We must stay ahead of the game to treat infection because of multiple routes the bacteria use to propel the evolution and spread of resistance.

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