Universal Impact Factor0.9285:2012; 1.2210:2013

Index Copernicus
ICV 2011: 5.09
ICV 2012: 6.42
ICV 2013: 15.8

NAAS Rating
2012 : 1.3;
2013-2014:2.69

Received on:
31st July 2015

Revised on:
20th October 2015

Accepted on:
28th October 2015

Published on:
1st December 2015

Volume No.
Online & Print
70 (2015)

Page No.
101 to 110

Life Sciences Leaflets is an international open access print & e journal, peer reviewed, worldwide abstract listed, published every month with ISSN, RNI Free- membership, downloads and access.

Review: ANTIMICROBIAL DRUG RESISTANCE IN THE PERSPECTIVE OF BACTERIAL MUTATION

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ABSTRACT:

Antimicrobial drug resistance (AMR) is a global challenge in treatment and prevention of infectious diseases in man and animals. It has great economic impact due to reduced productivity of life and higher cost of treatment involved. The major causes responsible for AMR are misuse, overuse or improper use of antimicrobials for human and veterinary diseases. Mutation is one of several mechanisms by which bacterial species develop resistance against antimicrobial drug. The bacterial genome mutation leads to either target modification or target alteration. The occurrence of mutation is influenced by several environmental factors as well as intrinsic characteristics of bacterial genome. Gene duplication or amplification and point mutation are type of mutation process responsible for alteration in genetic material of bacteria which provides AMR. Mutation is dynamic, random and variable in nature. It does not occur consistently throughout length of genome. Hypermutable loci showing high mutation rates have been identified in bacteria like *H. influenza* and *N. meningitides*. The mutation rate varies under different circumstances. Normal mutation rate in *E. coli* is 1 per 1010 nucleotides whereas in altered methyl directed mismatch repair system, the rate may increase up to 100-1000 fold times. The survivability and spread of resistant mutant after emergence is critical factor in determining clinical

outcome of antimicrobial usage. Intensity and length of duration of selection pressure determines the further survival and propagation of mutants. Mutation frequency and fitness cost are two important factor predicting development of resistance. These parameters provide clues on rate and overall quantum of mutation in bacterial cells. Mutation prevention concentration (MPC) indicates concentration of antimicrobial drugs that prevent the selection of first step resistant mutation in normal population of bacteria whereas mutation selection window (MSW) represents the range of antimicrobial concentration where the selection for resistant bacteria may occur. Mutation thus plays important role in conferring AMR. Understanding the process of mutation and its parameters would broaden future perspectives of research efforts on AMR and its clinical outcome.

KEY WORDS: *Antimicrobial drug resistance (AMR), Bacterial mutation, Mutation Prevention Concentration (MPC), Mutation Selection window (MSW).*

ANTIMICROBIAL RESISTANCE

Antimicrobial drug resistance (AMR) is defined as "the ability of microbes to grow in the presence of a drug that would normally kill them or limit their growth" (NIAID, 2009). Due to AMR, drugs become less effective in eliminating the infections from the body. As a consequence, many infectious diseases are now it is more challenging to combat them than they were just a few decades ago. As more microbes become resistant to antimicrobials, the options for treating the diseases they cause are reduced. This increasing AMR is a global problem for a broad range of microorganisms that threatens both human and animal health. Extensive, over-use and improper uses of antimicrobial drugs are among the factors that have contributed to the development of drug-resistant microbes. Antimicrobial resistance also causes huge economic losses globally due to reduced productivity caused by infectious illness of human beings and animals, as well as higher treatment costs (NIAID, 2009; WHO, 2015).

Bacteria have skill to survive in adverse environments that makes them to withstand in high acidic mediums or tolerate many more times radiation than that human can do. Rapid bacterial growth also exhibits characteristic rapid evolution and adaptation to different environments. This phenomenon can be seen only after few generations of growth under selection pressure, like use of antimicrobials, causing emergence of resistance as a natural outcome. This type of resistance cannot be prevented but can be minimized by rationalizing the use of antibiotics by carefully selecting the drug of choice, assessing the dosage duration and trying to adhere people to the standard treatment protocol (Martinez and Silley, 2010).

Inauspiciously, during past few decades wild use of antimicrobials in treatment of the infectious diseases results in rising number of resistant strains of bacteria and thus making treatment more difficult to be successful. Resistant form of commensal Gram negative bacteria like *Acinetobacter*, *Burkholderia*, *Klebsiella* and *Pseudomonas spp.* can emerge from patient's own microbial flora, poses additional problem along with common pathogens. These bacteria can possess multidrug resistance (MDR) or tolerance (Hawkey and Finch, 2006). Such a classical example of MDR bacteria in human is gut *Pseudomonas* spp. which has extremely potent natural efflux systems to pump different antibiotics out of the cell (Dean *et al.,* 2003; Cars and Nordberg, 2005). This type of bacterial resistance is example of intrinsic resistance *i.e.* mechanism is naturally encoded in its chromosome. These efflux pumps belong to the nodulation resistance division (NRD) family and they confer antibiotic resistance in many gram-negative bacteria and have a wide range of substrates, not only antimicrobials (Piddock, 2006).

In contrast, acquired resistance is often the result of horizontal gene transfer (HGT) of resistant gene through a mobile element 'plasmid' that carries resistance to one or more antimicrobials and is introduced from resist bacterium to the new host bacterium. Plasmids are capable of shuffling their gene content and acquiring new genes from the environment. Thus, these mosaic-like plasmids can be a combination of many circulating plasmids and carry multi-resistance genes responsible for increasing numbers of clinical outbreaks unresponsive to antimicrobials, *e.g.* the cases of carbapenem resistant New Delhi metallo-β-lactamase-1 (NDM-1) possessing strains of *Escherichia coli* and *Klebsiella pneumonia* (Kumarasamy *et al.,* 2010).

MUTATION AS A MEAN OF DRUG RESISTANCE

Naturally, mutations occur significantly and spontaneously in growing bacterial populations resulting in number of mutations in the bacterial genome. Many mutated bacteria failed to cope with its growing environment but some mutations sequentially provide bacteria an advantage to grow in changing environments. When such populations are exposed to selective condition *e.g.* presence of an antimicrobial, the bacteria with pre-existing mutations that are beneficial in that environment may be selected. These selected bacterial cells prevails the population and outgrow the bacteria without adventitious mutation (Snyder and Champress, 2007). Table-1 presents the mechanisms of mutational resistance for major antimicrobial classes (Kohanski *et al.* 2010). The bacterial genome mutation leads to either target modification or target alteration. For example, mutation in PBP gene leads to structural alteration of PBP and so beta-lactams fails to recognize PBP and yields resistance.

AMR in bacterial populations may arise by endogenous or exogenous mechanisms. Exogenous mechanisms involve acquired type of mutations involving HGT by plasmids and transponsons, or by recombination of foreign DNA into the chromosome; whereas endogenous resistance mechanisms involve the emergence of spontaneous mutations. Emergence of drug resistance through spontaneous mutations depends on environmental factors as well as characteristics of intrinsic bacterial genome (LeClerc *et al.*, 1996; Martinez and Baquero, 2000; Drlica, 2001).

Gene duplications/amplifications (GDA) are of the most common mutations found in the bacterial chromosomes and plasmids. GDAs are relatively easy to acquire. Interestingly, they are also easy to lose due to intrinsic instability, especially in the lack of selection pressure to maintain the duplicated region. But if the GDAs product gives higher fitness in available conditions for growth, it may be amplified and further augmented in the bacterial population (Sandegren and Andersson, 2009). About 10% of growing bacteria show duplications in their genome if grown in non-selective condition. These GDAs plays important role in generating adaptive responses to newer growth conditions and also paved basis for point mutations which are more permanent and stable genetic responses. Thus, GDAs are thought to be share significant part in AMR (Andersson and Hughes, 2009).

Gram negative bacteria like *Pseudomonas aeruginosa* and *Acinetobacter baumannii* includes point mutations in topoisomerase genes and regulatory mutations that increase the expression of intrinsic genes and operons (Rice, 2006). Point mutations are non-synonymous or synonymous changes in a single nucleotide. Nonsynonymous mutations means an observable mutation which alter an amino acid, whereas a synonymous mutation causes a change in the genetic sequence of DNA but results in the same genetic output or cause no change in the protein. Spontaneous mutation can also arise by the way of frameshift mutations, which can be a result of a deletion of a nucleotide or DNA fragment or by an intragenic insertion of mobilizable genetic material like transposon (Franklin and Snow, 2005; Snyder and Champress, 2007).

Spontaneous mutations do not make exclusive contribution to emergence and spread of drug resistance in bacteria. Conjugation, transformation and transduction are other mechanisms by which bacteria acquire additional genetic material containing resistance genes. Bacterial mutations that produce strains with AMR often come with some drawback known as fitness cost. This fitness or biological cost results in increase in generation times or decrease in bacterial virulence due to metabolic changes in the cell, thus transmission from one host to another becomes difficult. However, bacteria may overcome the biological cost by acquiring compensatory mutations that restore the fitness along with resistance (Andersson and Levin, 1999; Nagaev *et al.*, 2001; zur Wiesch *et al.*, 2010).

Mutations in bacterial genome are randomly variable in nature but not constant in all its parts. Genes closer to the ORI (origin of replication) of the chromosome shown lower rates of mutation compared to those away from ORI. Some bacteria like *Haemophilus influenza* and *Neisseria meningitides* have hypermutability loci showing high mutation rates. Mismatch repair systems and precision of the DNA polymerase maintains the fidelity of DNA replication and thus, lowers the probability of mutation. Heritable hypermutation in bacteria is mainly due to alterations in the methyl-directed mismatch repair (MMR) system (Jolivet-Gougeon *et al.,* 2011). All bacterial populations include mismatch repair-deficient individuals, and after mutations, confer resistance to an antibiotic. The strains that harbor these mismatch-repair mutations are called mutators and they are more reported in chronic bacterial infections where patient is under long antimicrobial therapy (Bjorkholm *et al*., 2001; Denamur and Matic, 2006).

Normal mutation rate in *Escherichia coli* is 1 per 1010 nucleotides following DNA replication, but alteration in methyl-directed mismatch-repair (MMR) systems can increase the mutation rate 100-1000 fold *e.g.* mutation in the genes encoding for proteins MutL and MutS (Denamur and Matic, 2006). Stress-induced mutagenesis involved mechanisms evolved by bacteria to increase their mutation rate in response to various stresses. DNA polymerase Pol IV under control of the LexA regulon involved in regulating this mutagenesis. In response to stress, transient increase in the mutation rate due to DNA polymerases V (*umuCD*) and IV (*dinB*) have also been reported for rifampicin resistance rates in *Salmonella typhimurium* (Martinez and Baquero, 2000; Koskiniemi *et al*., 2010).

Various factors influence the rate at which antimicrobial resistance (AMR) emerged in a bacterial population within the host *viz.* rate of formation of the resistant mutants, the biological cost of resistance, and the rate and pattern of antibiotic use. After the appearance of resistant mutants, their spread and maintenance are influenced by the rate and pattern of antibiotic use *i.e.* how much and how long the selection pressure is maintained, and the effect of the particular resistance on bacterial fitness.

MUTATION FREQUENCY AND MUTATION RATE

Mutation frequency and fitness cost are two important factors to forecast the development of resistance (Bjorkholm *et al*., 2001). Mutation frequency is the occurrence of a mutation in a specific time under specific conditions in one cell whereas mutation rate is an overall number of mutation events inthe whole genome independent of time and environment. This can be used in experimental evolution studies exposing bacteria to antimicrobials and counting the fraction that survived thetreatment. In the case of antibiotic resistance, the mutation rate is frequently defined as the *in vitro* frequency at which detectable mutants arise in a bacterial population in the presence of a given antibiotic concentration (Martinez and

Baquero, 2000). Baquero *et al.* (2004) studied polymorphisms in the rifampin resistance mutation frequency (*f*) in *Escherichia coli* strains and categorized them as follow: hypomutable ($\geq 8 \times 10^{-9}$); normutable (8×10^{-9}); weak hypermutable ($4\times10^{-8} \leq f < 4\times10^{-7}$) and strong hypermutable ($\geq 4 \times 10^{-7}$). LeClerc *et al.* (1996) reported about 0.1% of the natural *Escherichia coli* population as hypermutators whereas little higher incidence (0.7% strongly hypermutable) has been reported in clinical isolates (Baquero *et al*., 2004).

MPC (MUTATION PREVENTION CONCENTRATION)

Like minimum inhibitory concentration (MIC), mutant prevention concentration (MPC) can also be used to assess the *in vitro* antimicrobial susceptibility of a bacterium. MPC is the minimal antimicrobial concentration that prevents the selection of first-step resistant mutants in the presence of large numbers of cells (~10^9-10^{10} cfu) whereas MIC is the lowest concentration of an antimicrobial that will inhibit the visible growth of the tested organism where the standardized inoculum is ~10^5 cfu. Thus, MPC is the concentration that inhibits the bacterial growth plating higher numbers of bacteria which is more near to that found in actual clinical infections. MIC testing is required for evaluation of new antimicrobials and their potency toward pathogens and MIC values are used to verify the breakpoints for antimicrobials (Andrews, 2001). On the other hand, Usefulness of MPC is to prevent the selection and spread of resistant bacterial strains. Theoretically, MPC should prevent the rise of single-step resistant mutants. Antibiotics differ in their bactericidal activity (represented by MIC) as well as ability to prevent the selection of resistant mutants (represented by MPC). Low MICs do not necessarily predict low MPCs. Genetic resistance obtained through mutation provides the means for the bacteria to become resistant but it does not give guarantee to confer clinical resistance (Martinez *et al.* 2007).

MUTATION SELECTION WINDOW (MSW)

The MSW is defined as the region between two measures MIC and MPC, and represents the range of antimicrobial concentration where the selection for resistant bacteria may occur (figure 1). MSW can be advantageously used to predict and prevent the evolution of resistance in wild-type and single-drug resistant bacteria, which should be a parallel goal with curing the infection itself (Drlica, 2003; Zhao and Drlica, 2003; Blondeau, 2009). Low dosages of antibiotic drugs usually fall within the MSW, the drug-space within which resistance mutations can evolve and persist (Zhao and Drlica, 2002). Little is known about factors that affect the shape and size of the MSW for resistant bacteria, though drug-drug

interactions may play a role (Michel and Yeh, 2008), making multicomponent therapeutics a powerful tool for slowing the evolution of further resistance.

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Table – 1: Mechanisms of mutational drug resistance

Antimicrobial Class	Mechanism of action of antimicrobial	Mechanism of Mutational Resistance	Example
Beta-lactams	Disrupts the cell wall	Target modification	Mutations to PBP genes
Aminoglycosides	Inhibition of protein synthesis	Target alteration	mutations to proteins S12 and S5
Macrolides	Inhibition of protein synthesis	Target alteration	23S mutations
Quinolones	Inhibits nucleic acid synthesis	Target alteration	Mutations in Topoisomerase II enzymes

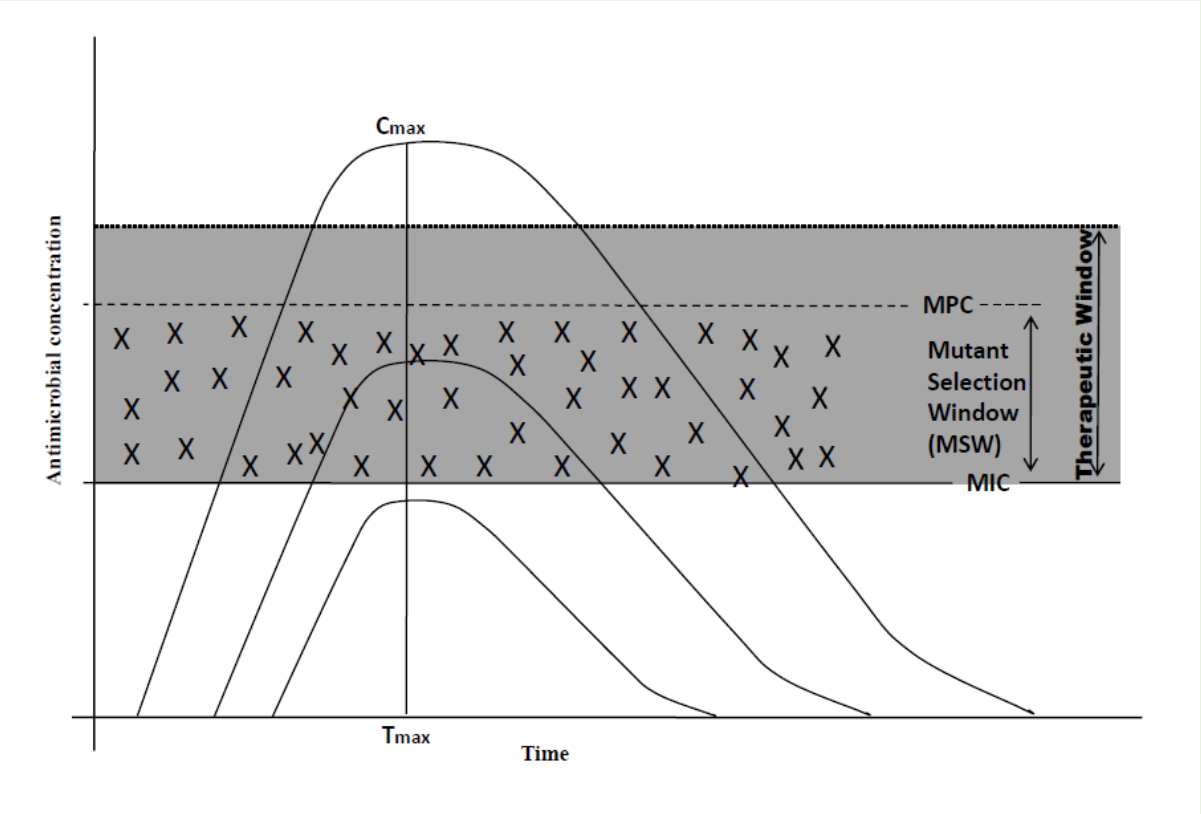

Figure 1: The relationship between MIC, MPC and MSW