Bacterial DNA Adenine Methyltransferase as a Novel Drug Target for Antibiotics: Current Status and Future Drug Discovery Challenges

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A B S T R A C T

Antibiotic resistance and the particular emergence of multi - resistant bacterial strains are clinically relevant issue involving serious threats to public health worldwide. DNA methylation, which changes the affinity and interaction of regulatory proteins with DNA, is an epigenetic mechanism that regulates numerous bacterial physiological processes, including chromosome replication, DNA segregation, mismatch repair, transposition and transcription. DNA adenine methylase (Dam), which methylates N-6 of adenine in the GATC sequence, plays a key role in the gene expression of bacterial virulence. Current antibiotic – resistant studies were gradually associated with adenine methyltransferase (DAM), an inhibitor of DNA, which plays a key role in the pathogenesis of bacteria. DAM is essential in regulating the replication and gene expression of the bacterium. The emergence of DAM in epigenetics studies facilitates the drug discovery of this multi-resistant pathogen. The goal of the review is to examine the status and challenges of the antibiotic resistance study in relation to bacterial DNA Adenine Methyltransferase.

Introduction

The epidemiology of multidrug-resistant (MRD) bacterial pathogens has become a global concern and according to WHO this pathogenic bacterial spread are threats to the human population leading to an increase of the mortality and morbidity rates(1). In 2014, economist Jim O'Neill projected that the mortality rate is around 700,000 deaths caused globally by the spread of antimicrobial resistance (AMR). Not only that, due to the increasing mortality and morbidity rate, it is estimated that the increasing number of AMR - related projects by 2050 are projected to lose

US$ 100 trillion, affecting the global economy (2). If the problem is not addressed, the number of deaths attributed to AMR is projected to be 10 million deaths per year. (3).

The prevalence of AMR infection would not only cost the research project, but would also affect labor that indirectly suppresses global economics. In a study carried out by RAND Europe, the estimated cost of AMR is conceptualized in the increase in mortality and morbidity rates (4). A substantial increase in AMR may incur an indirect cost. CDC outlined 18 threatening antibiotics resistant (AbR) classified into 3 groups depending on

the level of urgent threats, serious threats and threats. Three organisms notified as urgent threats are *Clostridioides difficile*, *Carbapenem - resistant Enterobacteriaceae* (CRE) and Drug - resistant *Neisseria gonorrhoeae*. (5).The drug - resistant bacteria that are already concerned are *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* and *Staphylococcus aurerus*, which are a group of CRE (3), (4).

Woodford(6) defines the term multi - resistant as being resistant to either more than two class drugs and extremely resistant to no more than two classes of drugs. The inclination of the AbR dissemination is due to the subsequent consumption of antibiotics, which develops the problematic strain of antibiotic pathogens (7). The evolution of (AbR) is described as enigmatic and, as described by Coque (8), AbR's expansion integrates with three mechanisms that are the evolution, invasion and occupation of these genetically related bacterial pathogens. The emergence of AbR involves ephemeral mobile - genetic elements and exploration of epigenetics (8).

One of the contributing factors to AbR's global expansion is the lack of antibiotic development (9). Although antibiotic development was first introduced in the early 1940s, the improper use of antibiotics contributed to the development of AbR bacterium. The main cause is the ability of the bacterial pathogens to adapt to the environment, hence the antibiotic - resistant mechanism rapidly developed. The clonal expansion of this pathogenic bacterium remains enigmatic until today. Carbapenems antibiotics are commonly used for severe infection caused by the CRE bacterium, whereas Colistin is only used as a last resort treatment provided there is no other empirical treatment available(10), (11). Carbapenems are the last β-lactams that retain antibiotics that are less toxic and highly effective while

colistin is highly toxic but most reliable to MRD pathogenic bacteria. However, the emergence of MRD uses the antibiotic - resistant mechanism that leads to clonal expansion (12).

In 2015, the WHO outlined plans to tackle the emergence of AMR, which includes increasing awareness to curb the prevalence of AMR, encouraging the use of medicines in human or animal health, drug development and vaccination. However, despite numerous awareness campaigns on the emergence of AMR to educate the public, the information alone may not be sufficient to address the problem. The highest priority is to understand the underlying mechanisms of the determinants towards the viability involved in the dissemination of AbR bacteria pathogens (13). The current approach to epidemiological studies includes the exploitation of the epigenetic mechanism that is responsible for the genetic attribute that causes AbR traits.

Due to the emergence of AbR bacteria, the need for new drug target identification is crucial as there is an increased prevalence of AbR infection. The development of CRE and colistin - resistant bacteria required the identification of potential drug targets in the development of antibiotics towards MDR bacteria. Most therapeutic targets focus on understanding the virulence factor but not on the viability that makes the target inhibitors unlikely to cause distortion to the host cell that develops the AbR mechanism. (14). The current approach to the reduction of the infection rate is not effective against the emergence of MDR requiring further chemotherapeutic findings of the new drug target.

According to Hoagland (15), the newly drug target agent is ideally to have the following element which one of it is the novel mechanism that is capable to attenuate cross

resistance. He also added that the characteristic fits best with other criteria, which include a restricted spectrum of activity and a small rate of emergence of spontaneous resistance. Current antibiotics are developed to target the cellular process of translation, transcription, replication and cell wall that makes the bacteria resistant to development or acquisition due to their intrinsic mechanism. (16). This becomes the hallmark for requiring an alternative auxiliary target for a new drug target.

The current drug discovery process

Experimental drug discovery

New drug target approach integrates with different pathways, in particular finding an alternative target that could help combat antibiotic resistance. Recent technological advancement approaches in - vivo and in - vitro experimental approaches to drug discovery of new drug targets and the analysis of the responsible determinants that trigger the bacteria resistant virulence and pathogenesis. The current drug discovery process suggested a potential new mechanism for AMR drug discovery focusing on alternative pathways of the underlying mechanism in the cellular structure of the AbR bacterial pathogens.

Current studies have shown that many antibiotics have been developed to combat AbR pathogens. However, some of the newly developed antibiotics are seen to be effective and cause side effects. Many approaches have been used to combat this AbR pathogen, including using old antibiotics. Colistin is an old antibiotic produced in 1950 and identified as the last resort antibiotics to treat bacterial infection due to its high toxicity. Despite the approach of using old antibiotics, however, it was reported that there was an increased prevalence of colistin E.coli resistance that

began to emerge in Vietnam in 2018. (10). A study conducted by Yamaguchi (17) confirmed the emergence of *Escherichia coli* a colisitin resistance gene of *mcr-1* and *-3* in ESBL in food samples in Vietnam.

Clofazimine, a new antibiotic used to treat MDR tuberculosis, has shown a positive indication against disease control. In a controlled randomized clinical trial in China, it resulted in about 73.6 percent of a patient infected with MDR tuberculosis of treatment success rate using clofazimine with the exclusion of HIV - seropositive (18). AbR pathogens pose a serious threat to a ventilator - associated pneumonia (VAP) in a patient in the Intense Care Unit (ICU). In a randomized controlled trial at a single centre, an observation was conducted to analyze the effectiveness of Aerosolized Amikacin (AA) for VAP therapy. It was concluded that the use of AA successfully eradicated MDR pathogens, but there were several limitations (19). Bedaquiline, a diarylquinoline, has also shown a positive culture conversion for XDR - TB patients(20).

Another interesting drug target mechanism under the drug discovery process is the regulatory mechanism of DNA adenine methyltransferase (Dam) in various pathogens(21). The association of Dam and the impact on the pathogenesis and virulence factor of a various organism is progressively demonstrated *in-vivo* and *in-vitro*. Some study has shown a profound finding between dam alteration and pathogenicity of pathogens. Either causing attenuation to the virulence or modulation resulting from overproduction, overgrowth or inactivation of the dam (Table 1).

Computer aided drug discovery

Computer aided drug design (CADD) through subtractive genomic approach is currently

integrated progressively in the current drug discovery process. This subtractive genomic is a newly developed approach in analysing and identifying novel drug targets of bacteria pathogens. Current clinical research involves a lot of costs and the estimated cost of an ABR - related research project is projected to increase annually and up to 2050. Computer aided drug design is a methodology based on bioinformatics that is more convenient and will not cost much time. The use of *in silico* subtractive genomic approach would facilitate in understanding the protein mechanism also an alternative approach in antibiotics discovery (28). This approach uses several available tools and databases (Table 2).

Some findings of the novel drug target of ABR pathogens using subtractive genomics approach are discussed and reported. Sarangi and Aggarwal (29) analyses a total of 1413 non homologous protein of *Neisseria meningitidis* MC58 which results in 9 potential protein that can be vaccine targets. Hossain (30) identified 11 protein Salmonella enterica strain ATCC BAA-1673 of essential protein with the broad - spectrum property of which FDA approved as druggable targets. Solanki (31) identified 52 out of 1578 proteomes of *Acinetobacter baumannii* potential drug target by performing a subtractive genomic approach which is then further analysed to only 2 suitable antigenic vaccine target.

Extensive research on subtractive genomics has been progressively integrated into CADD as this approach helps save time and cost. Along with this approach, some other CADD is the target for reverse vaccination and molecular docking. Molecular docking helps us understand the protein and ligand's active site where it can bind without consuming energy. Binding energy helps us determine the best inhibitor for either drug target, vaccine, or discovery. As the subtractive

genomics identifies the best protein or gene that has the potential to be a novel drug target or antibiotic target, the extended work from subtractive genomics can be further progress towards molecular docking or vaccine targeting. Molecular docking focuses on the protein structure and the chemical characteristic of the protein (Table 3). The protein that is used to perform molecular docking is also validated with some other software to validate the protein structure. Validation of the protein structure can be performed by using PROCHECK, Ramachandran Plot, PROSA and ERRAT. This is to generally see if the protein structure will result in good binding site and resolution.

DNA methylation as a drug target

Epigenetics is described as the changes that occur in the gene expression that is transferred to the daughter cells without alteration to the DNA sequence which involves several mechanisms(32). The field of epigenetics has progressively been explored to understand the underlying mechanism in drug discovery development. As described by Medina - Franco *et al.,* (33), epigenetics is divided into three main groups in which "writer" promotes the process of adding functional group to the protein, "readers" acting as macromolecules to function as the unit that recognizes and differentiates other foreign molecules and "erasers" that removes any alteration made by the writers by the chemical..

There are several mechanisms in epigenetics that involve the process of methylation of DNA. DNA methylation is known as gene expression control(34) and is transmitted by the DNA methyltransferases (DNMTs) enzyme(35). DNA methylation is crucial as the epigenetic control in both prokaryotes and eukaryotes. The process targets the DNA base adenine and cytosine that presents in both of

the organisms. The methylation process in eukaryotes is seen more impactful at the C5-Methyl-cytosine whereas for bacteria it can be found more at the N6-methyl-adenine(36). DNMTs main function is adding the methyl group from S-adenosyl-l methionine to either the base cytosine and adenine(37).

DNA adenine methyltraferase (Dam) enzyme are found in most enteric and other types of bacteria and carries several biological functions(38). The main role of the dam is to protect the host DNA against the digestion from the restriction enzyme endonuclease (32). In some studies Dam influences the viability of the bacteria which indirectly affects the virulence of the pathogenesis (38).Other biological function of Dam includes methyl-directed mismatch repair, gene regulation and chromosome replication (39), (40). The methylation process occurs at the GATC site of the DNA.

In the solution, Dam is a monomer that catalyses the process of which the methyl is donated from S-adenosyl-methionine (SAM) to the N6 position of the base adenine at the GATC sequence. Dam flips out the residue to the Dam catalytic site and modifies it. The substrate of the enzyme is hemi methylated DNA and at the GATC site which is configured behind the replication fork. Hemi methylated DNA is where one of the strands is methylated. At most cases, the parental strand is the methylated DNA and the process of the methyl transfer only occurs at the DNA strand that is newly synthesized (36), (40). Methylation helps to recognize between these two strands

Methyl- directed mismatch repair is a regulatory process whereby it recognizes the biosynthetic error during the occurrence of the replication fork. The hemi-methylated site differentiates between the template strand and the newly synthesized DNA allowing the

protein MutS to bind to the site where the mismatch occurs(32), (40). The binding of MutS promotes the process of the recruitment of the MutL and MutH to form a ternary complex (41).

Dam alteration also plays an important role in bacterial pathogenesis. The pathogenesis is either influenced by the deficiency or the overexpression that is said to cause attenuation which is the release of premature transcription in bacterium organism. Alteration of Dam that leads to attenuation was reported in *Salmonella typhimurium, Vibrio cholerae, Yersinia pestis, Yersinia pseudotuberculosis, Pasturella multocida, Caenorhabditis elegans,Haemophilus influenzae and Aeromonas hydrophila* (23), (24), (42)–(44). In a study by Mehling *et al.,* concluded that the Dam methylation in *Klebsiella pneumoniae* is partially attenuated (38).

The regulation of the virulence genes in *Escherchia coli, Salmonella and Yersenia* show strong indication of the association of DNA methylation which occurs at the post-transcriptional level (40). The alteration of Dam in virulence function causes *in vitro* effects either in phase variation, regulation of expressed gene *in vivo*, T3S, T2S, membrane instability, host cell invasion, motility, a decrease of the virulence property in animal model and oral live vaccine. The correlation of Dam system of the DNA methylation and the virulence of pathogens are caused by the pleiotropic effect (21).

Dam system can be targeted as novel antibiotics target as most of the drug development focuses on the virulence factor instead of the mechanism that sustain the viability of the pathogenic bacterium. The biological function of the underlying mechanism of Dam will make it as an interesting target of antibiotics which will

inhibit the Dam. There is a strong relation to the viability of the bacterial pathogens with the concentration of Dam. Novel antibiotics drug that targets Dam can be intriguing as the enzymatic activity is a lack in human. Inhibiting Dam by DNA methyltransferase inhibitors (DNMTi) can be detrimental to the bacterium. The inhibitors will reversely modify the deviating pattern of the DNA methylation by interfering the enzymatic activity of the DNMTs (46).

The S-adenosylmethionine (Adomet) dependant protein is the methyl donor that transfers the methyl group to N6 methyl adenine (47). Adomet is the most targeted post replicative modification of DNA which makes it the most potential source of methyltransferase inhibitors(45). Several natural bioactive chemical compound is found to act as the DNMTi such as curcumin, (-)-epigallocatechin-3-gallate (EGCG), mahanine, genistein, and quercetin despite not possessing high enzymatic based assay (33). The selection of potential inhibitory of Dam in a bacterial cell is preferably to be selective to the mammalian enzyme, does not cause toxicity, lack of non- specific interaction and the efficiency of the viability assay of the methylation-dependant (45). A diverse range of chemical compound can be screened based on the primary assay and enzymatic inhibition activity to identify new DNMTi. Some other compound that demonstrates a measurable preference for DNMTi are groups of the heteroaryl compound and the bicyclic heteroaromatic substituent (46), (47).

Future challenges

The epigenetics mechanism, Dam shows a strong evident in regulating the virulence of bacteria pathogens. Although it is shown that the phenotypic trait AMR bacteria pathogens shows correlation by alteration of the dam gene however understanding the phenotypic

changes could not be sufficient to combat with the emergence of AMR pathogens. In order to achieve a better understanding of the fundamentals of the DNA methylation as a regulatory process in bacterial pathogens, it is crucial to integrate both genomics and proteomics study.

A deeper understanding of epigenetics is required as a part of future challenges as an alternative auxiliary pathway involving the DNA methylation in various tissue and heritability of the genetic mechanism (48). Other future challenges include identifying potential Dam inhibitors by analysing bioactive chemical compound from a natural source and chemical derivatives which can be coherently studied with computational aided drug design.

Extensively, DNA methylation has been progressively targeted as an interesting drug target in other areas of studies like oncogenic, diabetes and other diseases. Thus, more studies should be focused on AMR bacterial pathogens.

In the future, an extensive study should focus on potential drug target for MDR pathogens. Taylor (49) suggested that targeting lipopolysaccharide and fatty acid biosynthesis small molecule combination therapy could be efficient against gram-negative infection. Anisimov (14) reported that derived inhibitors of aryl sulfamoyl adenosine to inhibit adhesion of *Yersinia pestis* could potentially be targeted to develop antibiotics.

In a study by Wellington (50) reported that there is a strong indication of the efficiacy of azetidine derivative BRD4952 by allosteric inhibition targeting tryptophan synthase (TrpAB) of *Mycobacterium tuberculosis(Mtb)* which can be detrimental to Mtb. Petchiappan (16) reviewed that inhibiting sRNAs and riboswitches by small molecule inhibitors and

peptide inhibitors of biofilms could potentially combat AbR bacteria. An extensive study towards finding a responsible mechanism for AMR is also focused on the DNA replication of the bacteria pathogens that helps to sustain the viability. Eijk (51) summarizes the development of antimicrobials that target the DNA replication protein; novel bacterial topoisomers inhibitors(NBTIs), DNA ligase inhibitors and DNA polymerase III inhibitors.

Although, there are many newly antibiotic that is a claim to eradicate the MDR pathogens without causing newly resistant expansion pathogen, there were lack of clinical trials and focuses on the current area. More clinical trial on antibiotics should be focused on to study the efficacy with a larger sample of the group. As evidently, Dam plays a vital role in regulating the virulence and pathogenicity of the AbR pathogens, more clinical trial and studies should be emphasized.

Table.1 *In-vivo* and *in-vitro* effects of alteration of Dam on various pathogens

Bacteria	Effects	Reference
Yersenia pseudotuberculosis	Attenuation of virulence	(22)
Vibrio cholera	Attenuation of virulence	(22)
Yersenia pestis	**Inactivation of Dam:** Distortion on the gene expression that results in an inclination of the number of genes expressed with SOS response which induce protection to the plague infection	(23)
Aeromanas hydrophila	**Overproduction of Dam:** causes a significant decrease of about 58% of the motility of the bacterium	(24)
Salmonella enterica serovar Typhimurium (S. typhimurium)	Modulation of the expression and the translocation of SPI-5-encoded *sopB* gene which is aids in bacterial invasion	(25)
Klebsiella pneumoniae	**Loss of Dam** causes a modulation of the pathogenicity, decrease of virulence.	(26)
Escherichia coli	**Methylation of Dam** regulates the replication origin	(27)

Table.2 Summary of subtractive genomic approach with available tools and databases

Tools/Databases	Functions	Website
UniProt	To obtain proteomic sequence	https://www.uniprot.org/
GenBank	To obtain genomic sequence	https://www.ncbi.nlm.nih.gov/genbank/
BlastP	To analyse the non-homologous protein sequence	https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins
Database of Essential Genes (DEG)	To analyse the essential gene or protein	http://www.essentialgene.org/
Kyoto Encyclopedia of Genes and Genomes	To identify unique pathways in the protein/gene	https://www.genome.jp/kaas-bin/kaas_main
PsortB	To analyse the subcellular localization of the protein/gene	http://www.psort.org/psortb/index.html
STRING	To analyse the protein-protein interaction	https://string-db.org/

Table.3 Some example of available software and database for identification of drug target

Tools	Function	Website
ExPASy ProtParam Proteomics	To analyse the physiochemical properties of the protein	(http://web.expasy.org/protparam/)
MODELLER	Use as a tool to perform homology modelling	https://salilab.org/modeller/
Computed Atlas of Surface Topography of Proteins (CastP)	To predict the active site of the protein	http://sts.bioe.uic.edu/castp/index.html?2was
PubCHEM database	To retrieve the ligand chemical structure	https://pubchem.ncbi.nlm.nih.gov/
Autodock Vina	To perform Docking	http://vina.scripps.edu/

In conclusion, the emergence of antimicrobial resistance causes a dynamic impact globally. The increasing bacterial resistant pathogen dissemination could prominently affect the mortality and morbidity of the human population. It is vital to understand the root of determinants of the spread through the importance of epigenetics mechanism. Current antibiotic target is not able to combat the antibiotic resistance leading to an urge to discovering an auxiliary pathway to understanding the underlying mechanism that

causes the resistant trait. Evidently DNA adenine methyltransferase can be an interesting target towards drug discovery especially in the development of antibiotics. Bioinformatics-based methodologies can be a new ideal approach of drug discovery as this can help to understand protein mechanism without consuming a lot of time also incurring a lot of costs.

Abbreviations

MRD-multidrug-resistant; AMR-antimicrobial resistance; AbR-antibiotics resistant; CRE-Carbapenem - resistant Enterobacteriaceae; Dam-DNA adenine methyltransferase

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