Asian Science Bulletin

OPEN ACCESS

Mechanisms and Possible Strategies to Fight Against the Antibiotic Resistance

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ABSTRACT

Antibiotic resistance (ABR) is a major concern for people nowadays. Apart from human-caused resistance, bacteria can develop self-resistance to antibiotics through a variety of mechanisms such as drug degradation, efflux, sequestration and target change. Due to resistance, the number of people infected with multidrug-resistant bacteria as well as the death toll is increasing every year which has led to enhanced economic cost. To ameliorate public health and keep our planet safe, it is high time to take proper action against the problem. Technological advancement could greatly assist in this aspect. Fragment-Based Drug Discovery (FBDD) and drug repurposing are such kinds of novel approaches that rely on advanced biophysical computational methods. While, FBDD uses a fragment library to identify potent molecules, drug repurposing mostly depends on the Food and Drug Administration (FDA) approved drugs. Another important strategy is iron chelation which is also an interesting way of fighting against ABR. This review emphasizes the importance of using advanced technology and novel approaches to address the problem in a more effective way. The combined effort of research for drug development and public health initiatives might be possible solutions to combat the growing antibiotic resistance threat.

KEYWORDS

Antibiotics, Methicillin-resistant *Staphylococcus aureus* (MRSA), Fragment-Based Drug Discovery (FBDD), repurposing, iron chelation, Nuclear Magnetic Resonance (NMR), Surface Plasmon Resonance (SPR)

INTRODUCTION

Antibiotic, a term that originated from "antibiosis" stands for "against life". Previously antibiotics were regarded as organic compounds of microorganism's toxic to other microorganisms. In recent times, this definition has changed with the introduction of antimicrobials that are produced by synthetic methods either partly or wholly[1,2]. Antibiotics are classified in many ways however, chemical structures, mechanism of action and spectrum of activity are the general way of classifying antibiotics[3]. The β-lactam, macrolide, tetracycline, quinolone, aminoglycosides and sulphonamides are some common classes of antibiotics that are classified according to their molecular structures[4,5]. On the other hand, inhibitors of the synthesis of bacterial cell walls, proteins, nucleic acid and antimetabolites are based on antibiotics' mechanism of action of antibiotics[6]. Antibiotics having similar structural classes will exhibit similar activity, toxicity and other side effects[2].

ISSN: 1813-0070 (Online)
https://doi.org/10.17311/asb.2023.31.46

Received: 15 Apr. 2023
Accepted: 30 Aug. 2023
Published: 4 Sep. 2023

Although the invention of antibiotics is one of the most important discoveries and saves the lives of millions of people every year, growing antibiotic resistance (ABR) is making antibiotic treatment more and more complex with the passage of time. ABR poses a severe threat to human, animal and environmental health. Infections remain a major source of illness and death on a global scale. The emergence of antimicrobial resistance has considerably worsened the effects of these infections, both in terms of the sheer number of cases and the financial burden on healthcare systems. Recently ABR has surged significantly while the development of new antibiotics has decreased[7-10]. It has a profound negative impact in the case of mortality as well as economic cost. It is estimated that every year the death toll reaches 700,000 globally due to ABR and this number is expected to reach 10 million per annum by 2050[11]. In the United States of America, every year 99,000 people lose their lives due to hospital-acquired infections (HAIs) caused by pathogens resistant to antibiotics. Back in 2006, pneumonia and sepsis were two common HAIs that caused 50,000 deaths with $8 billion in costs in the United States of America[12]. However, the predicted global economic cost due to ABR will reach $100 trillion in 2050[11].

Many of the bacterial pathogens responsible for human disease epidemics have developed into forms that are resistant to multiple drugs after exposure to antibiotics. The term "superbugs" refers to microorganisms that have become more harmful and deadly due to multiple genetic changes that give them high levels of resistance to antibiotics commonly used to treat them. As a result, the available treatment options for these microorganisms are limited, leading to longer and more expensive hospital stays. In some instances, these highly resistant strains have also gained a greater ability to cause severe illness and spread to others. In a practical sense, antibiotic resistance can be seen as a factor that contributes to the severity of an infection[13]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a well-known superbug and it was first detected in 1960. Although, vancomycin was effective against MRSA the MRSA superbug developed resistance against this antibiotic and vancomycin-resistant *Staphylococcus aureus* induced. Infection caused by MRSA is very common in many countries of Europe, America and many parts of the Asia-Pacific[14]. The death toll reached 11,285 because of MRSA alone in the United States of America[15]. Over time, MRSA has obtained prominent flexibility in the case of emerging and spreading in various epidemiological circumstances such as in health care settings, communities and animals which has been detected recently[15]. Infection-control systems particularly focusing on healthcare-Associated Infections (HAIs) are facing challenges due to MRSA infection[15]. Vancomycin-Resistant Enterococci (VRE) is another clinical challenge. In medical facilities and similar healthcare environments, enterococci bacteria cause infections in patients, which can then spread to the bloodstream, surgical sites and the urinary tract. *Enterococcus faecium* and *Enterococcus faecalis* are the predominant bacterial strains identified in documented instances. Despite Vancomycin-Resistant Enterococci (VRE) having a lesser occurrence and impact compared to MRSA, there are still approximately 54,500 cases of enterococci infections contracted within US hospitals each year. Infection rates differ among various types of enterococci, but Vancomycin resistance contributes to 30% of all healthcare-related enterococcal infections annually in the United States[16].

Streptococcus pneumoniae, commonly known as pneumococcus, stands as the primary culprit behind cases of pneumonia acquired outside of healthcare settings. It holds a significant status as a leading contributor to fatalities among children below the age of 5 on a global scale. Additional ailments resulting from pneumococcus infection encompass bloodstream infections, middle ear inflammation (otitis media, and inflammation of the membranes surrounding the brain and spinal cord (meningitis). When it comes to bacterial meningitis, pneumococcus is linked with death rates spanning from 16 to 37%. Among adult survivors, roughly 30 to 50% exhibit enduring lingering symptoms[17]. Encouragingly, a range of pneumococcal vaccines have been developed thus far, all demonstrating effective outcomes against various serotypes. Notable vaccines, including PSV23, PCV7, PCV10 and PCV13, have proven to be impactful defences against these resilient bacteria[18]. Drug-resistant *Mycobacterium tuberculosis* is another

threat to worldwide public health that caused 1.3 million deaths in 2012 according to a report published by WHO[19]. Individuals afflicted by strains of tuberculosis (TB) that have developed resistance to both isoniazid and rifampicin, termed Multidrug-Resistant (MDR) TB, are essentially untreatable using standard initial therapies. Currently, the persistent propagation of MDR-TB stands as one of the most pressing and formidable obstacles in the realm of global TB management. Back in 2012, there were an estimated 450,000 fresh cases of MDR-TB along with 170,000 associated fatalities. On a global scale, MDR-TB accounts for 3.8% of newly diagnosed TB patients and 20% of individuals with a history of prior treatment. The most elevated rates of MDR-TB are concentrated in Eastern European and Central Asian countries, where the MDR strains pose a substantial threat of becoming as widespread as the more susceptible strains of the disease.

In the year 2006, the phrase Extensively Drug-resistant TB (XDR-TB) was introduced to characterize strains of MDR-TB that show resistance to fluoroquinolones and second-line injectable medications. It is approximated that XDR-TB is present in 9.6% of MDR-TB cases across the globe. Initially, the surge in MDR-TB occurrences was believed to be fueled by transmissions occurring within healthcare settings, particularly among individuals who are HIV-positive[19]. Multidrug-resistant (MDR) *Pseudomonas aeruginosa is* a frequent source of HAIs and each year out of 51,000 healthcare-associated infections in the United States of America caused by *P. aeruginosa*, 6000 (13%) are due to MDR and among them 400 faces to death. A few strains of MDR *P. aeruginosa* have developed resistance to almost all available antibiotics include aminoglycosides, cephalosporins, fluoroquinolones and carbapenems[14]. *Acinetobacter baumannii*, a Gram-negative bacterium within the Moraxellaceae family, is chiefly responsible for infections contracted within healthcare environments. These infections span a wide range, encompassing conditions such as Hospital-acquired and Ventilator-associated Pneumonia (HAP, VAP), urinary tract infections, meningitis, bacteremia, as well as gastrointestinal and skin/wound infections. This bacterium poses a significant global health threat and presents a formidable challenge for treatment due to its ongoing emergence and escalating resistance. In 2018, The World Health Organization (WHO) designated Carbapenem-resistant *Acinetobacter baumannii* (CRAB) as the highest-priority target for antibiotic research and development[19,20]. In recent times, drug-resistant *Neisseria gonorrhoeae* has become a responsible factor for the disease gonorrhea which is transmitted sexually and the main characteristics of this disease are expulsion and irritation of the urethra, cervix, pharynx, or rectum. This disease can cause severe complexity in the reproduction process. According to the Centers for Disease Control and Prevention CDC, every year 800,000 new cases are seen due to gonorrhea which stands in the second position in terms of infectious disease in the United States of America[21].

Both the developed and developing nations are victims of the harmful consequences of ABR. However, the condition is not that much worse in developed countries compared to developing countries. Using antibiotics without a prescription, low-quality drugs, improper governance and utilizing surpluses antibiotics in cattle foods are some prime causes of developing ABR. Moreover, an unhygienic healthcare system, malnutrition, chronic and frequent infection and inability to have proper treatment are also playing a crucial role in this aspect. Moreover, decreased interest in investing in antibiotic research has made this more exacerbate[10,22].

In this review, the possible reasons and mechanisms of ABR with possible solutions to fight against this global problem have been discussed.

MECHANISM OF ANTIBIOTIC RESISTANCE

Development of self-resistance: Bacteria that produce antibiotics by themselves have several mechanisms used for self-protection against their produced antibiotics. They use several mechanisms at the same time to ensure the complete inhibition of the biological activity of the produced molecules so

that they can survive against the bactericidal effect. Surprisingly, the genetic materials inducing self-resistance are clustered in most of the cases altogether with the genes contributing to antibiotic biosynthesis and the expression of the genes being co-regulated[23].

Alteration or breakdown of antibiotics: Antibiotic alteration is a common way of decreasing the effectiveness of antibiotics for aminoglycoside, chloramphenicol and β-lactam antibiotics. A wide range of enzymes associated with aminoglycoside modification is available in bacteria that produce antibiotics and some of these enzymes are O-phosphotransferases and O-adenyltransferases participating in phosphorylation and adenylation of the aminoglycoside antibiotic. Although antibiotic modification enzymes (AMEs) were detected initially during the beginning of the 1970s in *Streptomyces* and their biochemical reaction was similar to antibiotic-resistant clinical strains, there is no direct relationship between the synthesis of aminoglycosides and AMEs in *Streptomyces* species. For instance, a species may not produce antibiotics but it still contains some AMEs and in other cases, the opposite scenario may be seen[24,25]. There is also an anomaly like in streptomycin resistance, in this case, a straight relationship has been found between the synthesis of antibiotics and AMEs for developing self-resistance. Here, streptomycin 6-phosphotransferase an enzyme synthesizing streptomycin plays a role in developing streptomycin resistance produced by *S. griseus* and the enzyme does it by converting the streptomycin to an inactivated form known as streptomycin 6-phosphate[23,26]. For self-protection, antibiotic modification has been observed for other groups of antibiotics like in the bleomycin (BLM) group of antibiotics and acetylation modifies the antibiotics. *Streptomyces verticillus* and *Streptoalloteichus hindustanus* produce bleomycin and tallysomysin, respectively. After acetylation, BLMs and TLMs become metal-free which prevents the proper formation of the binding of the metal domain of these antibiotics[27]. Another enzyme group, chloramphenicol acetyltransferases (CATs) participate in the acetylation of another antibiotic chloramphenicol and these enzymes are readily available in clinical strains[28]. Apart from the above-discussed modification process, resistance also develops to β-lactam antibiotics and in this case, an antibiotic-hydrolyzing enzyme β-lactamase is responsible. These enzymes abound in *Streptomyces* and these four types (A, B, C and D). This classification has been made according to the sequence of amino acids and the utilization of different catalytic ions[29]. A phylogenetic screening showed that except D, many *Streptomyces* species have enzymes of the other three groups but no information regarding the number of β-lactamases and the level of β-lactam antibiotic resistance to the *Streptomyces* species[30]. The probable reason behind this is that β-lactamases are produced simultaneously in most *Streptomyces* species and the production of these enzymes is not correlated to developing resistance or the biosynthesis of the antibiotics. The appearance of β-lactamases enzymes in the producer bacteria gives rise to a significant question regarding the coexistence of β-lactams and β-lactamases at the same time in the host bacteria. A possible explanation is that these enzymes do not have a crucial role in developing resistance but regulate the activity associated with penicillin-binding-proteins[31,32]. However, this will be discussed in the last section of the mechanism of antibiotic resistance.

Efflux of antibiotics: Another customary mechanism of developing self-resistance is antibiotic efflux although this process is accompanied by other mechanisms including antibiotic as well as target modification. A crystal-clear idea about the efflux mechanism has been detected in *Streptomyces peucetius* which is a producer of two crucial lifesaving anticancer antibiotics known as daunorubicin (Dnr) and doxorubicin (Dox). They exert their activity by preventing the replication of DNA by interacting with the DNA. DrrAB, a transporter of an ABC family participates in the effluxion of those antibiotics in *S. peucetius*. Moreover, the transporter is coded by rab genes which help in the biosynthesis of the mentioned antibiotics[33]. The DrrAB system has molecular and biochemical significance and deep studies have been done on it. The DrrAB pump contains dual subunits, one of them is DrrA and another one is DrrB. The DrrA subunit acts as a catalytic nucleotide-binding domain (NBD) while another subunit DrrB acts as a carrier protein and helps in the formation of the transmembrane domain (TMD). An experiment conducted with

inverted membrane vesicles showed that DrrAB was directly related to participating in the efflux mechanism in Dox[32]. In *S. peucetius,* the DrrAB is regarded as a devoted transporter of Dnr and Dox as it is located in the cluster of genes of Dox biosynthesis. An interesting fact has been revealed by research that the DrrAB system is a transporter for multidrug having extended specificity for different substrates. Moreover, it is capable of transporting MDR (multidrug resistance) pump substrates that were identified before[34]. Considering this, the DrrAB system has similarities to *P*-glycoprotein (Pgp) which is a multidrug transporter and a major cause of failure in chemotherapy due to its overexpression in the cancer cells[35]. Researchers have shown that in DrrB, several helices forming crucial aromatic residues contribute to generating sites where drugs interact. Aromatic residues are also used by the mammalian Pgp to obtain sufficient flexibility to recognize the substrates[34,36]. OtrC is another example of an efflux system contributing to developing self-resistance which is available in *Streptomyces rimosus* that produces oxytetracycline. Otrc shows multidrug specificity. Two efflux proteins OtrB (formerly known as TetB) and OtrC are responsible for the case of self-resistance in *S. rimosus.* OtrB and OtrC are found inside and outside of the biosynthesis cluster, respectively. OtrB is in the major facilitator superfamily (MFS) of transport proteins, however, little about the mechanism of action as well as substrate specificity of OtrB has been known till now[23]. OtrC belongs to the ABC family and it has a resistant nature to several antibacterial drugs and MDR like DrrAB and the resistant antibiotics include ampicillin, oxytetracycline, doxorubicin and vancomycin[23,37]. There remains a homologous relationship between DrrAB and OtrC and they exhibit high sequence similarities in the identified motifs earlier that include amino acids Asp (D)-Glu (E)-Ala (A)-Asp (D) (DEAD) and the Leucine (L), Aspartate (D), Glutamate (E), Valine (V), Leucine (L) and Phenylalanine (F) LDEVLF motif of DrrA and the EAA-like motif in DrrB suggesting a close connection among efflux systems available in various producer organisms[38,39]. It may be assumed that the efflux systems available in bacteria are particular to the antibiotics for which they are designed. The above-described two instances indicate that these systems can recognize polyspecific drugs. This scenario has raised significant questions. What is the necessity of a multidrug transporter required for antibiotic-producing bacteria? Where did the DrrAB-like polyspecific antibiotic and drug efflux system come from? Is there a link between maximal efflux systems and biosynthesis-regulating gene clusters? A possible explanation might be this those transporters responsible for the resistance to antibiotics might have originated from the general defence efflux system[40,41]. Such origin might be an explanation for the multi-specificity of these systems and their capability of adaptability to transport the produced antibiotics in the producer organisms. To get a clear concept of these questions, other available efflux systems should be analyzed[42]. Apart from the above-discussed antibiotics, lantibiotic, tylosin and actinorhodin are some other antibiotics that use the ABC and MFS transporters to develop self-resistance. Unfortunately, very little has been known about their molecular mechanism as well as substrate specificity[23,43-45].

Sequestration of antibiotic: Sequestration inhibits the activity of the drug-binding proteins which leads to the prevention of antibiotics from binding to the target molecule. The bacteria producing bleomycin classes antibiotics develop resistance by sequestering antibiotics after interacting with the protein TlmA, BlmA, ZbmA in *S. hindustanus* ATCC 31158, *S. verticillus* and *S. flavoviridis*, respectively[46,47]. Every member producing bleomycin-family antibiotics has more than one ABC transporter-related gene located in the biosynthesis clusters which are used to remove the drug-binding proteins so that the antibiotics cannot bind with them[48-50].

Target modification: Target modification has a profound influence on developing self-resistance to inhibit the action of different classes of antibiotics including β-lactam, glycopeptide, macrolide, lincosamides, streptogramin (MLS) and aminoglycoside. To treat bacterial infections, Penicillin and cephalosporins are the most frequently prescribed β-lactam antibiotics. In 1929, Alexander Fleming discovered penicillin for the first time[51] and it was revived in 1940 and 1941 by Ogawara[30] and Chain *et al.*[52], respectively. The structure of the β-lactam antibiotics is the same as the Penicillin-binding

proteins (PBP) substrates (peptidoglycan precursors) that induce acetylation of the serine residue to the active site leading to the inactivation of the antibiotics[53]. *Streptomyces* species are gram-positive bacteria that are strongly resistant to penicillin and this happens as a result of excessive production of PBPs or synthesis of PBPs having reduced affinity. A, B and C are three different types of PBPs commonly available in bacteria[54]. After analyzing the biosynthesis cluster of bacteria involved in β-lactam production proved that they usually have genes regulating the PBPs which contribute to the induction of self-resistance[54,55]. Surprisingly, over 10 PBPs with the A and B classes are available in *Streptomyces* species which is more compared to other *Actinobacteria*. Few PBPs among the available PBPs in the *Streptomyces* exhibit lower affinity for β-lactams and the predicted reason is not having a domain of serine/threonine-protein kinase (STPK) where β-lactams bind[56-58]. Vancomycin and teicoplanin are glycopeptide and lipoglycopeptide groups of antibiotics that exert their effectiveness to contain the activity of gram-positive organisms by interacting with the D-alanyl-D-alanine of the lipid II bacterial cell wall precursor and through sequestration of the lipid II substrate which leads to the termination of the peptidoglycan layer formation. These two antibiotics are the last option to treat MRSA and *Enterococci* species-induced infections. Developing bacterial resistance to these drugs is supposed to be a severe threat to public health[59]. Gram-negative bacteria have developed resistance against these antibiotics due to having the outer membrane which prevents the binding of these antibiotics with the target molecule[60,61]. Genes associated with vancomycin resistance were first identified in clinical strains from *Amycolatopsis orientalis*[62]. Biosynthetic gene cluster containing the ABC transporter as well as VanHAX (VanA cluster) resistance genes. The VanH dehydrogenase turns the pyruvate into d-lactate, VanA and VanX are d-Ala-d-Lac ligase and d-Ala-d-Ala dipeptidase, respectively which break down the residual d-Ala-d-Ala dipeptide that is responsible for developing resistance. Apart from vancomycin-producing *Actinoplanes teichomyceticus* and *Streptomyces toyokanesis*, VanHAX is also seen in *Streptomyces coelicolor* which does not produce vancomycin[63,64]. For vancomycin-related glycopeptide, gene clusters were cloned and ABC transporters were discovered in all of them. Furthermore, *Nonomuraea* species producing A40926, a glycopeptide antibiotic contains VanY genes instead of VanHAX genes that encode a new d,d-peptidase/d, d-carboxypeptidase inducing self-resistance[65,66]. Target alteration is also seen for the aminoglycosides group of antibiotics that are protein synthesis inhibitors and these antibiotics are divided into 4,6-disubstituted 2-deoxystreptamin (DOS), 4,5 disubstituted DOS and 4-monosubstituted DOS. These antibiotics have explicit effectivity after coupling with the helix 16S and 23S rRNA of the ribosomal subunit of bacteria that induces translational misinterpretation and stoppage of reactions associated with translocation[67-69]. Different genes are associated with developing self-resistance in different aminoglycoside antibiotics. *Actinobacteria* synthesize these groups of antibiotics and they must have protective systems to protect themselves against their own synthesized product since *Actinobacteria* are prokaryotes. Aminoglycoside 6'-N-acetyltransferase (kanM) and 16 sec rRNA methyltransferase (kmr) are present in the gene clusters of the Kanamycin biosynthesis which are responsible for developing self-resistance against this antibiotic. Moreover, efflux genes kanO and kanN and transporter protein genes kanS, kanR and kanQ are also present in the cluster[70]. In the case of gene clusters relating to gentamicin biosynthesis, four different genes diving into three different classes are related to self-resistance[71]. Finally, methylation is involved in developing resistance to MLS antibiotics which are also protein synthesis inhibitors like aminoglycosides. Moderate-level resistance induces after mono methylation while dimethylation is responsible for[72].

Human activities behind antibiotic resistance: Self-medication and overuse of antibiotics are actively contributing to developing resistance. When medication is taken personally or with the suggestion of a non-medical professional is known as self-medication. Advertisements of drugs on radio, television and other media are responsible for why people are taking medications by themselves. Increasing healthcare costs is also provoking people for self-medication since everyone does not have the capability to maintain the cost[73]. Due to COVID-19 which was declared as a global emergency back in January, 30 2020[74],

worldwide use of antibiotics has increased alarmingly. According to researchers at Washington University, during the first wave of the disease, Indian adults used 216.4 million doses of antibiotics with more than 38 million doses of azithromycin. In most of the cases, those antibiotics were used to treat viral mild to moderate infections. Researchers are concerned that such inappropriate use of antibiotics will surely enhance the possibility of ABR[75]. Moreover, the self-medication of antibiotics due to COVID-19 has made the situation worse for patients with high blood pressure, cancer and diabetes since they are vulnerable to COVID-19[76,77]. Antibiotics are commonly utilized as growth supplements in cattle in both the developed and developing worlds. Antibiotics are utilized in the realm of livestock agriculture, serving purposes such as treating diseases in animals and being administered at lower than therapeutic levels in concentrated animal feed. This is done to promote growth, enhance the efficiency of converting feed into body weight and prevent the occurrence of diseases[78]. Antibiotics used in cattle are taken by people through their food. It was almost 35 years ago when the transfer of resistant microbes from animals to humans was first observed. At that time, significant rates of antibiotic resistance were discovered in both farm animals and farmers' gut flora. The newly found result suggests that resistant microbes can make their way to consumers via products processed from meat[79]. Antibiotic use in agriculture has an impact on the microbiome in the environment. Antibiotics given to animals are expelled in urine and feces to the extent that they are widely disseminated through fertilizer and groundwater. The use of antibiotics in agriculture has a profound influence on the environment's microbiome. When animals are administered antibiotics, these drugs are excreted in their urine and feces, leading to widespread dissemination through fertilizers and groundwater. This practice is also responsible for resistance. Newly conducted research by a team at Porto University has reported finding that dog food available in pet shops could be another major cause of antibiotic-resistant bacteria. More than half contain *Enterococci* species among the tested sample while one-third of the strains showed multidrug resistance. Also, they were resistant to linezolid which is considered the last option drug when all other antibiotics failed. The raw dog food contained some drug-resistant bacteria that have similarities with those prevalent in hospitals in many countries of Europe. This trend of using raw dog food could speed up the spreading of resistant microbes[80]. Another crucial factor associated with antibiotic resistance is the unwillingness of the pharmaceuticals to develop new antibiotics and this is due to economic and regulatory barriers. The 15 out of 18 largest pharmaceutical industries have decided not to invest in the development of new antibiotics. Collaboration between pharmaceutical industries has reduced substantially over the passage of time which has ultimately reduced the research diversity on antibiotics. Moreover, academic research on new antibiotics has also fallen due to insufficient funding as well as collaboration with the pharma industry. Since the development and approval of new antibiotics have been reduced, the limited treatment option is available to treat infection caused by resistant microbes. From 1980 to 1984, 19 new antibiotics were approved by the FDA and this number reached only 6 in the 2010-2014 time period[78,79,81].

NOVEL APPROACHES TO FIGHT AGAINST ANTIBIOTIC RESISTANCE

Fragment-based drug discovery: During the past twenty years, technological advances have been made in drug discovery and Fragment-Based Drug Discovery (FBDD) is one of them. In drug development, FBDD has appeared as a substitute for the High-Throughput Screening (HTS) method and is used extensively for academic purposes as well as in industry. Pexidertinib[82], Vemurafenib[83], Venetoclax[84] and Erdafitinib[85] are some FDA-approved drugs discovered by FBDD. In FBDD, generally, a chemical fragment library is used to get potent small compounds having simple chemical structures and the molecular weight is normally less than 300 Da[86,87]. The main advantages of FBDD over HTS are higher hit rates and interactions of high quality. The fragment library contains thousands of molecules that are screened to get molecules that induce high-quality interactions with the target protein while the HTS library contains millions of compounds and the molecular weight is higher (300-500 Da) compared to fragment molecules[88]. Selection of fragment library, hit identification method selection, determination of the fragment-target complex structure, structure-activity relationship (SAR) analysis and determining strategies to get the

potent molecules from the fragment are the common stages involved in FBDD[89]. No specific rules are followed for the molecular weight and the molecules number to design a fragment library however, in most of the cases, the rules-of-three is suggested which means the molecular weight <300 Da, ClogP<3 and hydrogen bond donors and acceptors <3[90]. In general, researchers design the fragment libraries for their research in FBDD and it is not surprising that the molecular weight is more than 300 Da in some cases. However, a number of libraries are available on a commercial basis. It is possible to expand the fragment library with increased diversity since the screening could be done quickly and the compound number in the library is not a major issue in this aspect. In the FBDD, a potent compound was identified using a library having 800 molecules[91]. It is important to select and use the screening method properly in the FBDD since the binding affinity among the fragment and the target molecule is low (usually in micromolar to the millimolar range) which makes the initial detection literally challenging. So, the screening method should have sufficient sensitivity to identify weak interactions. In this case, biophysical methods are used due to their higher sensitivity. Nuclear magnetic resonance (NMR), thermal shift assay (TSA), surface plasmon resonance (SPR) and X-ray crystallography are some common biophysical techniques that are being used in the FBDD frequently. NMR spectroscopy is one of the most prominent instrumentations used in the FBDD that has enough sensitivity to detect fragments with wide binding affinity ranging from nanomolar to millimolar. The major advantages of NMR over other biophysical techniques are this method generates fewer false-positive results and screening of fragment mixture can be done[92-94]. Saturation transfer difference spectroscopy (STD) and Water-LOGSY are two common methods used for hit detection by monitoring signal changes. Moreover, binding affinity can be prioritized using these methods[95,96]. In the case of screening a fragment library, ^{19}F-NMR is a proficient option[92]. Since fluorine is absent in biological compounds, it is frequently utilized in drug discovery to enhance the characteristics of molecules because the absence of fluorine clear signals can be detected for the molecules. The attractiveness of this NMR spectroscopy in FBDD is that not only the compound mixture is screened but also to choose the appropriate hit compounds. Another useful technique in FBDD is surface plasmon resonance (SPR) which could be utilized to identify the binding specificity, binding affinity and thermodynamic properties. Apart from these, dissociation and association rate constant can be measured which is helpful to get a proper idea about the molecular interactions which is helpful in the lead-optimization stage since the molecular interactions provide information regarding the binding affinity and activity. Moreover, the structure-activity relationship (SAR) of a chemical compound is analyzed from the dissociation and association rate constant[97,98]. X-ray crystallography is another strong instrument used in the FBDD. Protein structure and complexes with higher resolution could be obtained using this tool. It has a profound impact on structure-based drug design. Till now, structural data have been attained from this method to discover many effective inhibitors[99-101]. The SAR and mechanism of a compound can easily be understood from the crystal structure obtained from X-ray crystallography. In the FBDD, this method plays a crucial role in hit detection as well as conformation[102].

Drug repurposing: Drug repurposing means using FDA-approved drugs to explore new clinical applications other than the existing application[103]. The possible advantages of drug repositioning have come from two concepts. Firstly, drugs may have some obscure pharmacological activities and secondly, few similarities in molecular pathways and/or genetic factors are seen in the case of many diseases. The second concept has been applied in cancer treatment where using drug repurposing has helped to obtain drugs for cancer therapy from other classes of drugs[104,105]. So, it is expected that this scheme could be useful in finding new drugs to fight against ABR. Drug repurposing mostly depends on using compounds that already get approval by the FDA or other compounds rejected by pharmaceutical companies but the experimental data is available. Empirical screening, unconventional screening and *in-silico* screening are some screening strategies used in this process[106]. Using FDA-approved non-antibacterial drugs having antibacterial properties in the cell-based models is a popular method for drug repositioning which is empirical in nature. In this case, molecules are selected without knowing their

mechanism of action (MOA) which is the opposite of the normal repurposing approach where knowledge about the disease or interacting capabilities and MOA of a drug are prerequisites before selecting a drug. Recent improvement in the HTS has turned empirical screening into a simple and popular screening process after utilizing the most innovative tools available in bioassay, robotics and computation. Since this screening method provides little information about the MOA of a repurposed drug, it has been regarded as a major obstacle in drug discovery by drug repositioning[106,107]. Another useful method for drug repurposing is the *in-silico* screening of chemical databases and through this process, it is possible to get novel lead compounds for diseases. Advanced computing systems and freely accessible compound libraries are making this process more popular in the field of drug discovery. This screening is classified into two categories, ligand- and network-based screening. Molecular docking is the most common approach in *in-silico* drug discovery. It helps to understand the binding strength as well as the interacting key residues between a protein and a ligand molecule. Moreover, the orientation of the molecule within the catalytic site of the target protein can be predicted[108]. This ligand-based approach is used for repurposing FDA-approved drugs to get lead antimicrobials. For instance, entacapone which is an anti-Parkinson's drug was revealed as an antimicrobial agent against MDR Mycobacterium tuberculosis due to having similarities in the binding site between human catechol-O-methyltransferase and the enoyl-acyl carrier protein reductase (InhA) of bacteria. InhA is an enzyme that takes part in the synthesis of fatty acids[109]. It was found that entacapone had the minimum inhibitory concentration (MIC) below the toxicity level and this was confirmed by the cytotoxicity model and human neuroblastoma cell line was used in this experiment which means entacapone could be used as a lead drug molecule against tuberculosis. Moreover, this ligand-based pharmacophore-modeling using approved drugs has helped to get lead compounds against MRSA[110], *S. aureus* inhibitors[111] and inhibitors of galactose metabolism and lipopolysaccharide biosynthesis in Gram-negative microbes that are drug-resistant[112]. Pre-screening has a crucial impact on drug repositioning and it helps in compound arrangement according to the *in-silico* properties. This process helps to avoid the screening of a complete database and the cost is comparatively less. PubChem[113], DrugBank[114] and ChEMBL[115] are some freely available databases that can easily be used for repurposing. On the other hand, system biology and bioinformatics methods are used in the network-based *in-silico* screening to make a direct comparison between the responses of the host to the pathogens as well as drugs. Methods that are used in this screening vary according to the complexity of the over-expressed and under-expressed genes in the biological system. Network-based computational screening is a recent adjustment for antimicrobial drug repurposing. An example regarding this is the research of Chavali *et al.*[116], in that research metabolic modeling was used to induce a series of 15 genes and 8 double genes combination that was related to the target for a tropical disease and *Leishmaniasis major* is responsible for the disease. The researchers found an association between the genes and 254 FDA-approved drugs based on the drug-target interaction.

Iron chelation: Iron is an important trace component for living organisms to maintain the appropriate cellular functions. For example, it has a profound impact on electron transfer, reactive oxygen species (ROS) are produced in this process. Iron levels should be under control in the body since apoptosis may happen due to superoxide radicals[117]. This metal is mainly deposited in the liver and spleen. Iron level in the body is regulated by a hormone generated in the liver known as hepcidin. During infancy, childhood and pregnancy, the required iron level is high. Duodenal cytochrome b converts the dietary ferric iron into ferrous iron. Iron is processed within the body in two ways. Firstly, it is stored in ferritin if not required by the body and secondly, during the demand, it is transported in the circulation system with the help of ferroportin1 and then binds with transferrin[118,119].

Iron is essential for several bacterial processes including host colonization, multiplication and infection. Bacteria can get host iron in two ways. One of them is known as siderophore-mediated iron acquisition and another one is a specific acquisition mechanism. With the help of the second method, can get iron

from host complex proteins including heme, transferrin and lactoferrin. Siderophores are bacterial extracellular molecules having a strong affinity for ferric iron and these molecules are secreted by several gram-positive and gram-negative microbes. These molecules shred iron from the host protein and then the siderophore-iron complex is formed with the help of a receptor known as the siderophore-specific receptor available in the cell surface of the bacteria[120]. Over the last decade, researchers have demonstrated that iron chelation could be a crucial option to fight against ABR. Since iron is crucial for bacteria reducing the iron availability at the infection site has an immense role in combating bacterial infections. Such a strategy for limiting the iron level is using chelating agents that prevent the uptake of iron by microbes by sequestering it. Iron chelation also strengthens the activity of antibiotics. For example, vancomycin in combination with iron chelation showed sufficient effectivity in mice infected by MRSA[121].

These approaches could be useful for combating this global problem. It is high time to take the initiative to do so because due to ABR, the death toll and overall expenditure are increasing day by day. This problem might be a nightmare for developing countries like Bangladesh where people spend a significant amount of money on the treatment of infectious diseases[122]. If ABR continues increasing, it will be difficult for those people to maintain their healthcare costs.

CONCLUSION

Since antibiotic resistance is a global problem nowadays, it is important to find novel strategies to fight against ABR. Fragment-based drug discovery, drug repurposing and iron chelation are such kinds of novel approaches that could be useful in this aspect. While FBDD and drug repurposing will help in finding new antibiotics, iron chelation will decrease microbial growth as well as enhance the antibacterial effect against drug-resistant bacteria when combined with antibiotics. Moreover, collaboration should be strong between the academic research group and biotech to strengthen antibiotic research. All these combined efforts could be helpful to combat antibiotic resistance.

SIGNIFICANCE STATEMENT

Antibiotic resistance poses a critical and escalating threat to global public health, resulting in a rising number of infections and fatalities each year. Bacteria's ability to develop self-resistance through various mechanisms exacerbates the problem, leading to increased economic burdens. Self-medication of antibiotics has exacerbated the situation. The overall situation has been worse due to the COVID-19 pandemic because millions of doses of antibiotics have been used to treat the illness. There are unique scopes of research that can be taken into account to ensure safety which can be developing precision antibiotics using modern technology. This review has focused on the reasons for antibiotic resistance and possible novel approaches that can be used to ameliorate the situation.

ACKNOWLEDGMENT

The authors are acknowledged by the anonymous reviewers for their suggestions to improve the quality of the paper. The author M. Kamrujjaman acknowledged to University Grants Commission (UGC) and the University of Dhaka, Bangladesh.

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