Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2023, 15(8):01-12

Research Article

ISSN: 0975-7384
CODEN (USA): JCPRC5

A Systemic Review on Bioinformatics Tools and Approaches to Combat the Antibiotic Resistance

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Received: *05-Apr-2021, Manuscript No. JOCPR-23-30831;* ***Editor assigned:*** *08-Apr-2021, PreQC No. JOCPR-23-30831 (PQ);* ***Reviewed:*** *22-Apr-2021, QC No. JOCPR-23-30831;* ***Revised:*** *04-Aug-2023, Manuscript No. JOCPR-23-30831 (R);* ***Published:*** *01-Sep-2023*

ABSTRACT

Antibiotic resistance is a significant issue worldwide as overexposure of antibiotics in the form of excessive and unauthorized prescription by physicians or other medical personnel, the antibiotics added in food and fodder of animals in agriculture and aquaculture, etc. took a crucial role in the development of resistance genes against antibiotics as we can easily withstand by microbial residents of the soil, water. Our microbiota possesses such genes that can resist the activity or destroy the administered antibiotic molecules entirely and gradually turns into a serious matter. Hence, it is very essential to cultivate new classes of drugs. However, billions of dollars and longer than a decade have been invested in fulfilling this need. But, only a few antibiotics were approved and reported in the past decades. Thus, with the aid of applied bioscience disciplines, humanity has developed a new approach known as bioinformatics, which provided crucial routes for sculpting a natural living cell system and proteins, allowing scientists to discover operational drug strategies to combat the spread of antibiotic resistance among common infectious diseases by examining and elucidating a large amount of data (such as nucleic acid and amino acid patterns, protein domains, protein configurations and protein-ligand solid interaction behavior) at the molecular scale without the use of laboratory studies. Thus, in this article, we will withstand the application of bioinformatics tools and approaches to combat antibiotic resistance with the help of case studies.

Keywords: Antibiotic resistance, Bioinformatics, Antibiotics, Peptidoglycan, Multidrug resistance bacteria

INTRODUCTION

The conflict between humans and pathogenic organisms is very historical to humanity as we have been suffering from such diseases without any proper alternatives in the form of medication, but on the other hand, the discovery of the very first antibiotic agent, namely 'penicillin' by Alexander Fleming in the year 1928 provided the ray of hope to fight such disease. A steady control over the rate of infectious diseases was well achieved. Meanwhile, various antibiotics and antimicrobial agents of different classes were discovered to fulfill the same purpose. However, the first case of antibiotic resistance against penicillin was discovered in 1940 and subsequent cases of antibiotic resistance against various antibiotics have been reported since then.

As per the current scenario, the discovery of antibiotics has imparted in the direction of the procurement and escalation of resistance genes in bacteria and they segregate through horizontal gene transfer and 16s rDNA that in turn acts as the natural origin of various bacteria and makes themselves Multidrug Resistance (MDR) [1]. As per the study, excessive and unauthorized prescription and antibiotics exposure in food and fodder in agriculture and aquaculture play a significant role in antibiotic resistance [2].

Consequently, at present, humanity is struggling against infectious diseases. We are seeing an increase in cases of patients dying in hospitals for standard reasons such as microbial infections. Therefore, a sudden rise can be witnessed in demand for novel antibiotic production as the consumption level is highly lifted in 2010 [3]. Only a few antibiotics have been approved by the Food and Drug Administration (FDA) in recent decades, despite billions of dollars and more than a decade of research and development. Since the 1990s, though, there has been a significant vacuum in exploring new antibiotics. [4].

As per the statistical analysis by Centers for Disease Control and Prevention (CDCP) intelligence reports published in 2018, around 2,000,000 complaints have been registered against bacterial infections annually with single or multiple antibiotic resistance properties in the USA. The death toll estimation is close to 23,000 people [5]. Globally, it is predicted that these infections will lead to the deaths of 10 million people/year by 2050 [6]. Although an enormous sum of money (in billions) and time has been invested, only a few antibiotics have been sanctioned for prescription by the Food and Drug Administration (FDA) in the past decades. The expenditures endorsed on treating these resistant infections vary from around $7,000 to $29,000 for every patient. For example, medical expenses related to Methicillin-Resistant *Staphylococcus aureus* (MRSA) infections in the United States are around $18,000 per case, nearly € 9,000 per case in Germany and over 100,000 Swiss francs per case in Switzerland [7].

So, the primary purpose is to raise issues against this kind of bacteria and find some alternative measures to tackle this issue either by physical means or using the contribution and collaboration of applied bioscience disciplines. In this article, we will focus on scientific approaches. Several scientists, like Paulien Hogeweg and Ben Hesper, have discovered the "Bioinformatics" approach by investing some time and investment. We can model a cellular living organism system and protein structure in many fundamental ways using this approach, which aids in discovering effective treatment strategies to overcome this diverse problem. It can also introduce nucleotide and amino acid fragments, protein structures and the behavior of associated proteins ligand interaction at the molecular level [8].

MATERIALS AND METHODS

Antibiotics and its bacterial targets

Selman Waksman coined the term "antibiotic" in the year 1942 to label the antagonistic action of any substance produced by a microorganism, excluding synthetic compounds to kill or prevent the growth of other (pathogenic) microbes. In the current scenario, the term 'antibiotics' denotes any prescribed medicine that is utilized for the treatment against pathogenic microbes that in turn acts *via* killing (bactericidal) or inhibits the growth (bacteriostatic) of bacteria regardless of their fabrication that is produced by a microorganism or developed scientifically in a laboratory. The terms ‘antibiotic’ and ‘antimicrobial’ are often used interchangeably but are not synonymous because antibiotics are originated from microbial means (such as penicillin), whereas “antimicrobial” refers to synthetic fabricated compounds which terminate microbial environment [9].

However, both bactericidal and bacteriostatic activity (as discussed earlier) mainly depends upon the target selectivity of antibiotics, e.g., β-Lactum antibiotics are mainly suited for obstructing the cell wall synthesis, Quinolones inhibit the action of DNA gyrase, Sulfonamides are responsible for inhibition of PABA (Para-Aminobenzoic Acid) (puss) a metabolite formed by bacteria, etc. However, the target selectivity of antibiotics will be well depicted in Table 1 and Figure 1 [10].

Table 1: Antibiotic target selectivity.

Sr no	Antibiotic and its class	Drugs	Primary targets
1	Fluoroquinolones	Nalidixic acid, ciprofloxacin, levofloxacin and gemifloxacin	Topoisomerase II (DNA gyrase), topoisomerase IV
2	Trimethoprim-sulfamethoxazole	Co-trimoxazole (trimethoprim plus sulfamethoxazole in 1:5 ratio)	Tetrahydrofolic acid synthesis inhibitors
3	Rifamycins	Rifampicin	DNA-dependent RNA polymerase

4	β- Lactum antibiotics	Penicillins (penicillin, ampicillin, oxacillin), cephalosporins (cefazolin, cefoxitin ceftriaxone, cefepime) and carbapenems (imipenem)	Penicillin-binding proteins
5	β- Lactamase inhibitors	Salbactum, clavulanic acid, etc	β- Lactamase enzyme
6	Glycopeptides and glycolipopeptides	Vancomycin, teicoplanin	Peptidoglycan units (terminal d-Ala-d-Ala dipeptide)
7	Lipopeptides	Daptomycin and polymixin B	Cell membrane
8	Aminoglycosides	Gentamicin, tobramycin, streptomycin and kanamycin	30S ribosome
9	Macrolides	Erythromycin and azithromycin	50S ribosome
10	Streptogramins	Pristinamycin, dalfopristin and quinupristin	50S ribosome
11	Phenicols	Chloramphenicol	30S ribosome

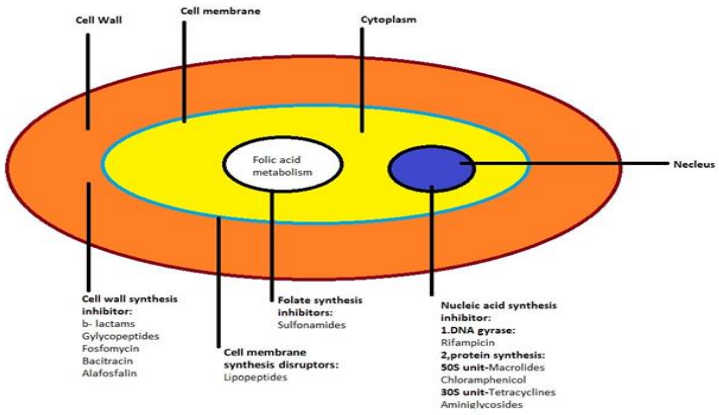

Figure 1: A well illustrative figure depicting how different classes of antibiotics act on different bacterial components.

The mechanism associated with antibiotic targeting the bacterial cell predominantly involves:

Injury to cell membrane/depolarization of cell membrane: The primary role of the cell membrane is to regulate the transfer or diffusion of a wide range of tiny molecules and new proteins in between intra- and extracellular areas. The cell membrane comprises roughly equal proportions of integral proteins and lipids, mainly phospholipids, in the asymmetric bilayer [11]. The zwitterionic Phosphatidylethanolamine (PE) and phosphatidylglycerol (Cardiolipin, CL) phospholipids, as well as the anionic Phosphatidylglycerol (PG). Glucolipids (mono- and glucosyl diacylglycerol), positively charged lysyl Phosphatidylglycerol (lysyl-PG) and anionic Phosphatidic Acid (PA) are often commonly found in minor amounts [12]. There are three main functions associated with asymmetric lipid bilayer present in the cytoplasmic membrane entails:

- To provide charge and physical form of the lipid species.
- To provide fluidity to the cell membrane.
- To provide selective permeability.

The foundation step that is associated with the interference in the structure of cell membrane *via* antibiotics mainly involves:

- Disruption of zwitterionic Phosphatidylethanolamine (PE) present on the membrane (Polymyxin B).
- Oxidative phosphorylation by forming pores in the cellular membrane (Valinomycin).
- Depolarization of cell membrane by releasing potassium ions from cytoplasm to extracellular membrane (Daptomycin).

- By dislocating divalent cations (*i.e*., Ca^{2+} and Mg^{2+}) from the phosphate groups of membrane lipids (Colistin).

Injury or interruption in cell wall synthesis by complete inhibition: The chief component that co-exists in both gram positives and gram negatives is Peptidoglycan (PG) (also recognized as murein), a long polymeric chain that comprises of two glycan subunits [N-acetylmuramic acid (MurNAc) and N-acetylglucosamine (GlcNAc)] are cross-linked *via* robust, flexible and short peptide bridges that are attached at MurNAc residue (in alternating fashion) [13].

Biosynthesis of bacterial cell wall

The biosynthesis of the bacterial cell wall is divided into three significant stages:

- The first stage comprises synthesizing sugar-coupled nucleotide associates, *i.e*., UDP-MurNAc-pentapeptide and UDP-N-acetylglucosamine (UDP-GlcNAc) inside the cytoplasm.
- Within the cytoplasm, substrate lipid metabolites are blended in the second step. UDP-MurNAc-phospho-MurNAc-pentapeptide pentapeptide's moiety is passed to the surface acceptor bactoprenol, resulting in the formation of lipid I. Then GlcNAc from UDP-GlcNAc is attached to lipid I, resulting in lipid II, which is also the polymerization reaction site in bacteria with specifically cross-linked PG.
- The polymerization of the recombinant disaccharide-peptide unit occurs outside of the cytoplasm through Penicillin-Binding Proteins (PBPs), accelerating the transglycosylation and transpeptidation processes lead to the formation of the PG's glycosidic and peptide bonds, accordingly.
- Transglycosydation: It forms the basis of glycan fibers at the lower end of MurNAc's promising lipid-binding PG that transferred to C-4 carbon glucosamine lipid residue linked to PG, with the corresponding release of undecaprenyl-pyrophosphate. Undecaprenyl-pyrophosphate is then dehydrated by dephosphorylated to produce the lipid carrier bactoprenol, found in the second cycle of synthesis.
- Transpeptidation: In this process, the D-Ala-D-Ala bond of a single stem peptide begins to separate and forms an enzyme-substrate intermediate through the corresponding release of the D-Ala terminal glycosidic formation.

A specific class of antibiotics linked with the interference of cell wall synthesis usually includes β- Lactum antibiotics. These antibiotics act *via* hindering the transpeptidation reaction by binding at the transpeptidase domain as an analog of acyl-D-alanyl-D-alanine. Thus, cell wall integrity is lost as the glycosidic linkage is incomplete; thus, the cell wall becomes fragile and results in cell death.

To protect against such hindrance, as mentioned earlier, many species of gram negatives and gram positives came up with an alternative in the form β-Lactamase enzyme. These enzymes can inactivate β-Lactam antibiotics by degrading the amide bond of the β-Lactum ring. This, resulting in the discovery of a new class of antibiotics, namely β-Lactamase inhibitors, which acts by inactivating β-Lactamase enzyme. Usually, these antibiotics are prescribed in the combination eg; Amoxicillin and clavulanic acid.

Disturbance in the biological metabolic pathway by complete inhibition: Antibiotics that disrupt bacterial metabolic pathways are usually denoted as anti-metabolites. This agent acts *via* competitive inhibition to prevent the formation of the enzyme-substrate complex by functioning as a structural analog of substrate that binds with the specific enzyme to prevent the catabolism process for the formation of active biological metabolite. Eg., Sulfonamides in combination with trimethoprim (SMZ-TMZ) creates hurdles in the detachment steps of DNA and RNA precursor synthesis and protein, where sulfonamides portray structurally analogy with P-Aminobenzoic Acid (PABA) and trimethoprim, illustrates structurally analogy with Tetrahydrofolic acid (THF).

Inhibition of nucleic acid synthesis

For inhibition of DNA synthesis: Fluoroquinolones such as levofloxacin act against enzymes involved in bacterial DNA synthesis is DNA gyrase or topoisomerase IV, respectively. Generally, Fluoroquinolones (FQ) acts *via* the inhibition of these enzymes. For instance, FQ acts *via* interfering in the formation of replication fork by DNA gyrase. FQs bind with both the subunits to prevent this reaction, *i.e.,* gyrA that carries out DNA nicking and gyrB, which introduces negative supercoils, respectively.

For inhibition of RNA synthesis: Antibiotics like rifampicin (*i.e*., anti-TB drug) act on enzymes, namely DNA-dependent RNA polymerase, principally involved in the transcription of DNA to mRNA. Rifampicin usually acts by binding to a β subunit of RNA polymerase within a DNA/RNA channel (but far from the active site) that inhibits the formation of RNA-DNA helicase extracts from the DNA template by violating hydrogen bonds produced in between.

Inhibition of protein synthesis

Protein synthesis is a multi-step process that usually incorporates many enzymes and the coordinates of mRNA translation with the help of tRNA in the ribosome. Antibiotic objectives associated with inhibition of protein synthesis mainly include:

- Inhibition of 23S rRNA for the prevention of aminoacyl tRNA binding towards (A) site (aminoacyl) site of ribosome (Chloramphenicol, Tetracyclin, aminoglycosides, etc.).
- Inhibition of 23S rRNA for the prevention of aminoacyl tRNA binding towards (E) site (exit site) of the ribosome (Erythromycin).
- By preventing binding or relapse of EF-G (translocase) enzyme, which is responsible for translocation of polypeptide tRNA from A to P site (peptidyl) (Thiostrepton, fusidic acid, etc.).

RESULTS AND DISCUSSION

Bacterial resistance against antibiotics: A major concern

Antibiotic resistance refers to the ability of a microbial species or other microorganisms to survive, reproduce or performing other functions that are essential for their survival at any doses of the same antibiotics in any dosage form is administered that was previously found effective against them (Figure 2).

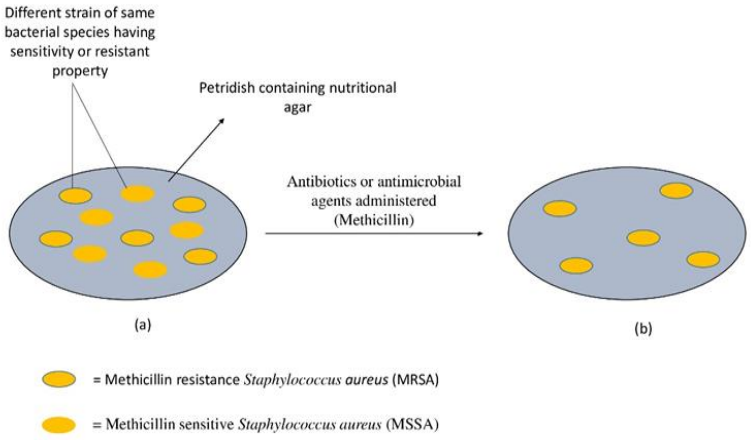

Figure 2: Illustration of how bacteria of the same species can or cannot be resistant against common antibiotic or antimicrobial agent.

Types of antibiotic resistance mechanism

There are mainly four types of mechanism by which resistance against antibiotics develop these includes:

Natural (intrensic) resistance: It may be due to either intrinsic (*i.e.*, always conveyed in bacterial species) or induced factors (bacteria naturally inherit, *i.e*., the genes; however, they are only expressed after exposure to antibiotics at resistance levels). Intrinsic resistance can be denoted as some unanimously pooled characteristics within the bacterial species and are devoid of prior contact to the antibiotic. Genetic factors usually cause this type of resistance. E.g., *Mycobacterium tuberculosis* is naturally resistant to β-Lactum antibiotics as its cell wall is devoid of peptidoglycan.

Acquired (genetic) resistance: This kind of resistance arises due to changes in genetic characteristics of bacteria that mainly include mutation or *via* acquiring any genetic material by other bacteria that possess antibiotic resistance genes (horizontal gene transfer) in chromosomal and extra-chromosomal structures present in bacteria.

Mutational resistance (resistance *via* chromosomal means: This type of resistance arises when the affected number of viruses undergoes genetic mutations that affect the function of the drug, leading to the survival of a stored cell in the

presence of an anti-bacterial molecule. As soon as an allergic reaction occurs, the antibiotic eliminates the affected area, and the resistant bacteria begin to thrive. On the other hand, these mutations are more costly for cell homeostasis (e.g., low fitness) and are only maintained when needed in the presence of antibiotics. Physical (UV radiation) and chemical factors are generally involved in the occurrence of such resistance.

Generally, mutations bring about antimicrobial resistance by the following mechanism:

- Adjustments of the antimicrobial target
- Dropping the level of the drug inside the bacterial cell
- Triggering efflux mechanisms to emit destructive molecules such as antibiotics, or
- Modulation of regulatory networks exists in metabolic pathways

Horizontal Gene Transfer (HGT) (resistance *via* extra-chromosomal means: The most important drivers associated with the evolution of antibodies are the purchase of foreign DNA material using Horizontal Gene Transfer (HGT). They often manage to control the development of antibodies. The primary source of anti-bacterial genes for clinically relevant bacteria is the "Environmental resistome." In addition, this genetic mutation has contributed to the spread of many commonly used antimicrobials.

Characteristically, bacteria procures genetic matter from an external source *via* conversion (incorporation of naked DNA), transduction (phage mediated) and conjugation (bacterial "sex"), but conjugation is the most often used method for the emergence of the genetic basis of antibiotic resistance. It involves contact between bacterial cells with the help of Mobile Gene Elements (MGE), *i.e*., plasmids, transposons and integrons.

Cross-resistance: It refers to the resistance phenomenon when structural similarity has been found between two classes of antibiotics. The same can be observed in completely unrelated antibiotic groups due to chromosomal and extra-chromosomal factors-Eg, the resistance between penicillin and cephalosporin and resistance between erythromycin and lincomycin, respectively.

Multidrug Resistance (MDR) and Pan-Resistance (PR): Multidrug resistance bacterium talks on the point of the microorganism strains that are immune to three or additional categories of antimicrobials. Suppose these strains are immune to about one or 2 antibiotic classes. In that case, they are considered extensively drug-resistant and if the strain is immune to all accessible antibiotics, then they're classified as pan-resistance. Such resistance occurs due to the accumulation of multiple genes encoding resistance to a single drug that is usually present on-Resistance (R) plasmids and secondly may be due to increment in the expression of genes that encodes for multidrug efflux pumps, enzymatic inactivation, changes in the target structure, etc (Table 2).

Table 2: Resistance mechanism of bacteria concerning the different classes of antibiotics.

Drug/drug class	Mechanism of bacterial resistance
Penicillins and cephalosporins	ß-lactamase inactivation; acylase and esterase modification; elimination of outer membrane proteins; alteration of penicillin-binding proteins
Monolactums	Enzymatic inactivation by ß-lactamase
Carbapenems	ß-lactamase inactivation; elimination of outer-membrane proteins
Vancomycin	Inhibition of glycopeptide access
Trimethoprim	Dihydrofolate reductase production increased, as well as trimethoprim-insensitive dihydrofolate reductase production
Sulfonamides	P-aminobenzoic acid production increased; pteridine production increased; sulfonamide-insensitive dihydropteroate synthetase production increased
Aminoglycosides	Acetylation, phosphorylation and nucleotidylation of enzymes; ribosomal alteration; decreased drug absorption
Chloramphenicol	It reduced compound permeability due to enzymatic inactivation by acetylation
Macrolides	Esterase-mediated enzymatic modification of 23S ribosomal RNA
Lincosamides	Nucleotidylation or phosphorylation of enzymes; modification of 23S ribosomal RNA
Tetracyclines	Chemical alteration precedes active efflux

Quinolones	Drug permeability is reduced due to a mutation in DNA gyrase subunit A

Biochemical or mechanistic basis of antibiotic resistance: According to Darwin's theory, the existence of different species on this planet is majorly dependent on how the individual organism adapts to changes based on the environmental factors in which they are living. In short, 'survival of the fittest is the main motto of this theory. As we can predict how well the species of *Staphylococcus aureus* has adopted some genes which can resist the activity of methicillin and made them methicillin-resistant. Likewise, many bacteria have also adopted some of the sophisticated mechanisms of drug resistance that majorly includes multiple biochemical pathways to evade both bactericidal and bacteriostatic activity of antibiotics or antimicrobial agents by millions of years of evolution and these evolutionary parameters are dependent upon environmental factors in which they are surviving. Eg., there are two species of *Staphylococcus aureus* based on adaptability of resistance against methicillin and vancomycin and thus make them methicillin and vancomycin-resistant *Staphylococcus aureus*, respectively. (MRSA can or cannot be resistant against vancomycin or vice-versa depending upon environmental factors). To provide universal classification regarding mechanisms of antibiotic resistance, we have categorized the biochemical path of antibiotic resistance (Figure 3); these comprise of several mechanisms like:

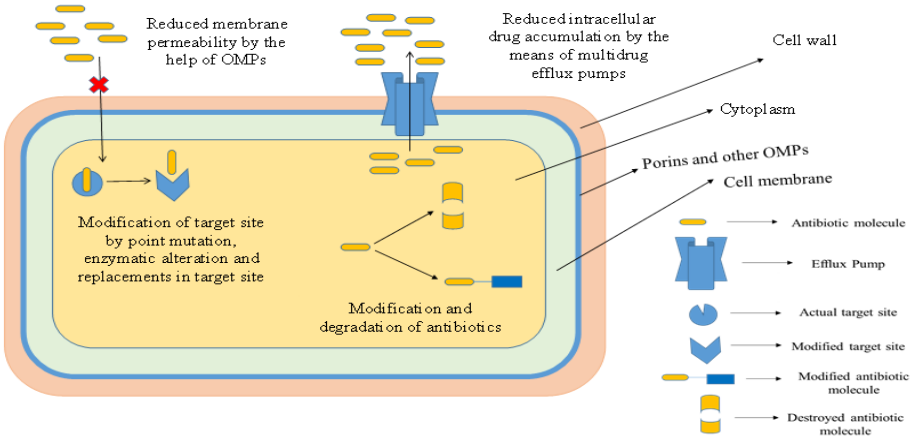

Figure 3: A well illustrative figure depicting how several bacterial species have been developed several resistance mechanisms for survival against various classes of antibiotics.

Deviations in target sites

Protection of target site: The responsibility of safeguarding the possible antibiotic or antimicrobial target sites has been predominantly established *via* coding of genetic determinants in bacterial chromosomes carried *via* Mobile Gene Elements (MGEs) that mediate target protection. The antibiotics that come under the class of tetracyclines, fluoroquinolones and fusidic acid are greatly affected by such mechanism and proteins such as Tet (M) and Tet (O) (tetracyclines), Qnr (fluoroquinolones) and FusB, as well as FusC (Fusidic Acid), respectively are mainly involved in fulfilling such mechanisms. To comprehend such mechanisms in detail, one of the best-considered examples involves tetracycline determinants like Tet (M) of *Streptococcus spp*. and Tet (O) *Campylobacter jejuni*, which has been widely circulated and originated in plasmids and wide varieties of transposons present in numerous bacterial species. This protein belongs to the superfamily of GTPase, which acts as a homolog against elongation factors, *i.e*., EF-G and EF-Tu, respectively, that aids in the synthesis of macromolecule-like proteins. Both Tet (M) and Tet (O) interacts and displaces the tetracyclines in a GTP-dependent fashion at its binding site that is domain IV of the 16S rRNA and tetracycline binding site of the ribosome. Furthermore, this interference helps in altering the ribosomal conformation by preventing the rebinding of antibiotics.

Modification of target site

To decrease the antibiotic affinity at the antibiotic binding site, modification of antibiotic target site is one of the best approaches, which can be accomplished *via* involvement of three tactics which comprises of:

Mutational alteration of target site: As discussed earlier, both physical and chemical factors are involved in mutation in the bacterial genes present in chromosomes, resulting in some changes regarding target structure. One of the

conventional, e.g., to understand the development of resistance *via* mutation of the target site is Rifampicin (RIF) resistance. The primary purpose for the discovery is to provide resistance against DNA dependent RNA polymerase, *i.e*., an enzyme with an α2ββ'σ subunit present in *M. tuberculosis*. The pocket assembly of RNA polymerase (encrypted *via* rpoB gene) is located at the β subunit that facilitates the binding of RIF that disrupts the transcription process by directly blocking the path of emerging RNA. On the other hand, a single-step point mutation in the rpoB gene has resulted in the substitution of amino acid. Several other genetic changes have also been reported, like providing the continuation of the transcriptional process by sparing the catalytic activity of the polymerase.

Enzymatic alteration of target sites for different classes of antibiotics: The enzymatic alteration of target sites can be well illustrated *via* CFR-induced linezolid resistance. The CFR gene is a plasmid endured contributing factor that encrypts for the enzyme named CFR enzyme, which belongs to the family of S-Adenosyl-L-Methionine (SAM) methylase, which is capable of providing resistance against phenols, lincosamides, pleuromutilins and streptogramin A.

Complete replacement and bypass of target site: This section will withstand some strategies of "bypassing antibiotic targets" by bacterial species. The approach involves adapting some kind of variations in the pre-existing target structure without hampering its biochemical function to avoid any interruption in the ongoing process. E.g., acquisition of exogenous PBP (penicillin-binding protein). Modifications in peptidoglycan structure *via* van genes clusters adapted against vancomycin by vancomycin-resistant enterococci and avoid inhibitory effects of antimicrobials by overproduction of antibiotic target to devastate the antibiotic by rapid increment in the number of targets e.g.; Trimethoprim-Sulfonamides (TMP-SMX).

As we all are familiar with the capabilities of β-lactam antibiotics *i.e*., to cause some kind of interference in the progression of bacterial cell wall synthesis by directly inhibiting Penicillin-Binding Protein (PBPs), which is responsible for the completion of both the process, *i.e*., transpeptidation and transglycosylation respectively. To prevent such interactions, bacterial species like Methicillin-Resistant *Staphylococcus aureus* (MRSA) has procured foreign genes, namely mecA, inside MGEs that in turn encrypts for PBP2a that doesn't allow some class antibiotics like β-lactam antibiotics like penicillins, cephalosporins (except for last generation compounds) and carbapenems to fulfill their purpose by completely modifying its structure that in turn results in unrecognition of target site.

Inactivation of antibiotic molecules

This strategy typically implicates fabrication and utilization of such enzymes whose resolve is to either disable or destroy the administered antibiotic molecules. As a consequence, the interaction between antibiotics and their target sounds unbearable. These are discussed in detail with several illustrations [13].

Chemical alteration of antibiotics: The enzymatic construction is gifted by several chemical modifications against antibiotic molecules *via* several methodologies embraced by both the bacterial classes. These enzymatic adjustments employ their mechanism of action against antibiotics that interferes with the synthesis of proteins at the ribosomal level. Consequently, numerous enzymes are designated and their biochemical reactions that catalysis chemical modifications that involve acetylation, phosphorylation and adenylation. The conclusive report states that steric hindrance is often responsible for the decrement in the avidity in drug-target (enzyme) interaction that has been redirected by higher MIC values. For instance, Aminoglycoside Modifying Enzymes (AMEs) generally exist in Mobile Gene Elements (MGEs). These enzymes function *via* covalently transforming hydroxyl or amino groups of aminoglycoside molecules and these genes encoding for AMEs have become a part of chromosomes in certain bacterial species. Based on the structure of the antibiotic molecule, these enzymes are classified as:

- Acetyltransferase [ACC].
- Adenyltransferase [ANT] or
- Phosphotransferase [APH].

Destruction of antibiotics: The chemistry behind the action of β-lactamases is the destruction of the amide bond that is available in the β-lactam ring by hydrolysis. The genes that encrypt for β-lactamases are keenly originated in bacterial genetic material or confined in MGEs as an additional genome fragment and are generally regarded as bla followed by its name (blaKPC). So far, as per the investigational studies, around 1,000 different β-lactamases have been designated and are still on the verge of discovery. These are collectively regarded as Extended-Spectrum B-Lactamase (ESBL) and these are categorized into two main classes, namely;

- Ambler classification: It usually relies on an amino acid sequence that aids to serve as an identity mark that separates β-lactamases into 4 groups (A, B, C, and D).

- Bush-Jacoby classification distributes β-lactamases into 4 sets, each with numerous subgroups as per the biochemical function they possess and substrate specificity.

Significantly, these two classification system does not fully overlap with each other, such as Ambler Class A and D comprised of enzymes according to group 2 and group 2d in Bush-Jacoby classification, respectively.

Ambler class A (group 2): They possess serine residue as a cofactor. Utmost, all enzymes of this class are inhibited *via* clavulanic acid, monobactams, excluding cephamycins (cefoxitin and cefotetan). These enzymes include TEM-1, SHV-1 (present in penicillinases and both hydrolyzes only penicillins), CXT-M and KPC (present in carbapenemases and they are responsible for the resistance against imipenem, meropenem, amoxicillin/clavulanate, etc.).

Ambler class B group (group 3): These enzymes are also referred to as metallo- β-lactamases that utilize metal ions (mostly Zn^{2+}) as a cofactor to attack β-lactam ring *via* nucleophilic reaction. These enzymes are responsible for the resistance against almost all classes of β-lactams, including carbapenems, etc. On the other hand, they have inhibited *via* ion chelating agents (EDTA). There are around 10 types of Metallo-β-lactamases, out of which four of them are most commonly found, namely IMP, VIM, SPM and NDM.

Ambler class C group (group 1): This class of β-lactamase enzymes are only concerned with the resistance against all the penicillins and cephalosporins, including cephamycins. The most common enzyme that comes under this class is Amp C β-lactamase enzymes.

Ambler class D group (group 2d): It generally involves Oxacillin Hydrolyzing Enzymes (OXAs) and the most commonly found enzyme belonging to OXAs are OXA-11, OXA-14 and OXA-16, respectively, apart from OXA-18 as they are resistant against β-lactamase inhibitors.

Reduced membrane permeability: To exert antibacterial activity against gram negatives, the compound must penetrate the cytoplasmic membrane. Still, then again, the manifestation of the Outer Membrane (OM) in these gram-negative bacteria reaching the intracellular or periplasmic targets seems to be dreadful as the primary function of the outer membrane is to limit the influx of the foreign molecule (antibiotics) from the external milieu. For instance, the action of such as β-lactams, vancomycin, tetracyclines and some fluoroquinolones are greatly exaggerated *via* variations in outer membrane permeability (as there are hydrophilic) *via* water-filled diffusion channels named 'porins' to cross the barrier. The porins are categorized as per their structure, choosiness and the regulation of their expression. The general e.g. of porins, which mediates porin-mediated antibiotic resistance, involves OmpF, OmpC, PhoE (*E.coli*), and OprD (also known as D2 protein) present in *P. aeruginosa* besides changes in porins can be expressed *via* a shift in the type of porins expressed a change in the level of porin expression and impairment of the porin function. Also, these can be observed in other bacteria like *N. gonorrhea*, *A. baumanii*, etc.

Reduced intracellular drug accumulation using multidrug efflux pumps: Efflux pumps refer to the bacterial gears that are gifted enough to expel any compounds or metabolites found to be lethal to the bacterial cell and push them out of the cell. This system is generally categorized into substrate-specific that is produced to expel out the specific or particular class of antibiotics and multidrug specificity that is frequently located in Multidrug-Resistant (MDR) bacteria. This kind of resistance is usually capable of disturbing the mechanistic approach of numerous antimicrobial classes: Protein synthesis inhibitors, fluoroquinolones, β-Lactams carbapenems, polymyxins, etc. The encoded genetic material for these efflux pumps is generally preserved and located in MGEs or chromosomes. Based on the investigational studies, the efflux pumps are categorized into five prominent families, namely;

- Major Facilitator Superfamily (MFS)
- Small Multidrug Resistance Family (SMR)
- Resistance Nodulation Cell Division family (RND).
- ATP Binding Cassette family (ABC)
- Multidrug and Toxic Compound Extrusion family (MATE)

The classical example associated with efflux-mediated resistance is the Tet efflux pump, responsible for inhibiting tetracyclines using protons as a source of energy. At present, a diverse amount of tet genes are sheltered in MGEs and are commonly found in gram negatives excluding Tet (K) and Tet (L), as they primarily originate in gram positives. These pumps originated from Tet genes that majorly affect tetracyclines and doxycyclines but are devoid of decreasing the susceptibility of minocycline or tigecycline, respectively.

Biofilm formation: It refers to a thin layer formed on both abiotic and biotic surfaces with the help of bacterial agglomerates that exists as well as mount together in public and are enclosed within a protective and adhesive Extracellular Polymeric Matrix (ECPM) of Exopolysaccharides (EPS), extracellular DNA (eDNA) and proteins. Furthermore, these bacterial agglomerates can interact with each other and the surrounding environment. To achieve the formation of biofilm, these bacterial agglomerates have to accomplish these three fundamental steps that are;

Adhesion: This step mainly employs attachment to any feasible surface by these agglomerates according to the suitable environmental conditions and safeguards towards the site.

Growth and maturation: This step is principally concerned with the formation and progression of micro-colonies. It is initiated *via* the generation of Exopolysaccharides (EPS) at the Extracellular Polymeric Matrix (ECPM). Afterward, we can withstand the increment in the progression of micro-colonies can be seen and thus resulted in the formation of multilayered cell bunches.

Detachment
It is divided into two types:

Active: It is usually originated by bacteria themselves with the help of quorum sensing and enzymatic degradation of the formed biofilm itself.

Passive: It is commonly triggered *via* peripheral influences like fluid shear, scraping and human intrusion.

The critical property associated with the formed matrix provides a barrier capable of providing a mechanical and biochemical shielding against antibiotics by lowering pH, oxygen and water availability and increasing carbon dioxide content. Thus, the probability of antibiotics against the bacteria is nullified. The most common pathogens found in biofilms in a healthcare setting are *S. aureus, P. aeruginosa, A. baumannii*, and *K. pneumonia*.

Bioinformatics, an only hope against superbugs: Bioinformatics refers to the fusion of computers, statistics and molecular biology for scheming and erection of software tools to fulfill various purposes like gaining, storing, investigating and spawning data associated with molecular biology to encounter new research in current molecular biology and medical research. The bioinformatics approach proceeds with selecting phytochemical compounds or we can utilize synthetic compounds from different informative databases for the same and are then subjected to virtual screening for the target evaluation and the methodology associated with designing new drug moiety with the help of bioinformatics tools. We will understand these steps in detail by utilizing phytochemicals as a source of drug.

Step I: An in-depth understanding regarding utilization of effective compounds from medicinal plants moieties for research studies, principally involves the collection of evidence regarding phytochemical constituents can be collected *via*:
- Literature review and reports
- Utilization of phytochemical compound database of medicinal plants

However, innumerable compound catalogs have been developed and are utilized for the sole purpose of reporting several characteristics and functions of phytochemical constituents.

Step 2: Afterwards, pre-treatment of these phytochemicals are performed by prediction of drug-like (druggable) properties, *i.e.*, ADME/T (Absorption, Distribution, Metabolism, Excretion and Toxicity) for the eradication of false drug compounds.

Step 3: In the next step, *in silico* virtual screening of these selected compounds based on ADMET is done with the help of combining theoretical aspects, which are well depicted in Table 3.

Table 3: Various theoretical aspects involved *in silico* virtual screening and some examples regarding the same.

	Virtual screening approaches		
Parameters	**Pharmacophore modeling**	**QSAR**	**Molecular docking**
Principle	Statistical approach	Graphical approach (Structural property *vs.* biological activity)	Molecular mechanics and quantum mechanics
Overview	This method is generally based on the establishment of the pharmacophore model. It also establishes the degree of similarity amid 3D conformations amid ligands and pharmacophore models to develop pharmacophore models	This approach focuses on identifying molecular descriptors that provide some inference, hypothesis, or assumptions about the action of molecules in an environmental, physicochemical, or biological system that is being studied using a numerical value	This method usually deals with the knowledge of the receptor and its structure. It also deals with its binding position and mimics the interactions between receptors and ligand molecules
Merits	High accuracy and proficiency, along with a variety of commercial or unrestricted pharmacophore databases, are available	With little or no previous laboratory evidence on operation, be capable of predicting the actions of a wide variety of compounds	A variety of commercial or unrestricted molecular docking software is available along with fully-fledged algorithms
Demerits	This methodology is effected *via* two processes: The quality of the pharmacophore model and the number of protein crystals	Does not have a thorough understanding of the mechanism of biological action, and there is a possibility of making highly inaccurate predictions about pharmacological or biological behavior	Increment in workload due to a large amount of data calculation of the massive amount of data available and
Softwares	GASP, Discovery studio, Apex-3D, SEAware, DISCOtech, etc.	Swiss ADME, ADMET SAR, pkCSM, etc.	AutoDock, Gilde, DOCK, FleX, ZDOCK, eHITS,etc.

Step 4: Interpretation of results from *in silico* virtual screening process is made to predict whether the particular compound is eligible for *in-vitro* and *in vivo* studies against the particular disease that has been targeted for the well-being of the society.

Application of bioinformatics against superbugs: A case study

Evaluation of novel curcumin derivatives against Methicillin-Resistance *Staphylococcus aureus* (MRSA) (2017): This article aimed to compare the activity of newly derived and synthesized curcumin compounds with curcumin and vancomycin against PBP2a present Methicillin-Resistance *Staphylococcus aureus* (MRSA) by utilizing *in silico* bioinformatics approaches like molecular docking followed by evaluation of molecular properties, bioactivity and drug-likeness and toxicity prediction of these derivatives by the help of *in silico* tools followed by *in vitro* studies like standard well diffusion method in Muller Hinton Agar media. In this study, preparation of ligand and proteins was successfully carried out by extraction of target protein structure (PBP 2a) from Methicillin-Resistance *Staphylococcus aureus* (MRSA) (PDB ID: 1VQQ) with the help of PDB database followed by the construction of 25 derivatives novel derivatives of curcumin by the help of ACD labs Chemsketch v 12.0. The next step mainly involves molecular docking with the help of GOLD (genetic optimization of ligand docking) software v.3.0.1 that operates principally on Genetic Algorithm (GA). Before that, the binding site of PBP2a was identified using the CASTp (Computed Atlas of Surface Topography of proteins) server. Among all of these curcumin derivatives that are constructed, the examination outcomes associated with derivative no 11, 16 and 20 were found to be very promising by displaying better docking fitness values. Thus, molecular, physicochemical and biological properties were determined using molinspiration cheminformatics online server displaying better docking scores. Furthermore, these compounds were subjected to the

prediction of toxicity utilizing the Osiris software. The validation of anti-MRSA activity was additionally confirmed *via in-vitro* studies *via* Muller-Hinton agar well diffusion method. In this method, the inhibitory activity of the promising candidate from all the 25 derivatives was compared with parent compound curcumin and vancomycin. Out of the selected derivatives *i.e*. 11, 16 and 20, 1E, 6E)-1-(4-hydroxy-2-chlorophenyl)-7-(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione (derivative no 11) showed best docking fitness value than other derivatives including curcumin and vancomycin. Thus from the results, we can predict that curcumin derivatives (especially derivative no 11) can also act as an inhibitor of PBP2a alongside curcumin and vancomycin.

Carbapenemase and efflux pump functions in carbapenem-resistant gram-negative bacteria are inhibited by quercetin (2020): The purpose of this article was to demonstrate and evaluate the ability of quercetin inhibitors, unlike carbapenemase and efflux pumps using an enzyme inhibition assay and efflux pump inhibition assay. Similarly, inhibitory actions at the cellular level were tested for various forms of *in-silico*, especially cell suspension. To conduct these studies, adverse bacterial strains of grams such as carbapenem-resistant *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. From cellular studies, it has been found that a stable structure between quercetin and carbapenemase has been developed that can withstand the superimposition of quercetin against meropenem, quercetin proximity to the nucleophile. The release of similar amino acids out of meropenem and quercetin-showed a contradiction between quercetin and meropenem binding to the ligand.

CONCLUSION

In contrast, quercetin was trapped in the center of the drug-binding site of the AcrB site of the PaβN efflux pump without contraindication. On the other hand, the adequate release of Gibb's free energy was released. Thus, the stability of quercetin and PaβN predicted the inhibitory potential of quercetin against the efflux pump. Similarly, assay studies regarding inhibitory action of carbapenemase with crude periplasmic extracts proved significant inhibition of meropenem *via* hydrolysis, and the results of fluorescence-based spectrophotometric measurement of intracellular ethidium bromide accumulation proved that quercetin has reduced the activity of overexpressing AcrB efflux pumps up to half the MIC and overexpressing mexB and adeB were not significantly inhibited by quercetin. For conducting molecular docking studies, PDB IDs of different variants of enzymes were utilized. These are as follows; 5OE0 (OXA-181), 4K0X (OXA-23), 3SPU (NDM-1), 5YD7 (VIM-2) and 3DW0 (KPC-2), respectively.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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