New directions in the fight against antibiotic resistance
Direcții noi în lupta cu antibiorezistența

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Rezumat

Antibioticele au transformat medicina, permițând tratamente eficiente pentru infecții fatale în trecut și sprijinind progrese importante, cum ar fi chirurgia și transplanturile. Totuși, rezistența tot mai larg răspândită reduce eficiența acestora, iar descoperirea de noi medicamente devine crucială în lupta continuă împotriva patogenilor umani. Antibiorezistența reprezintă una dintre cele mai mari provocări ale medicinei moderne, având un impact semnificativ asupra tratamentului infecțiilor bacteriene. Dezvoltarea rezistenței la antibiotice face ca tratamentele standard să devină ineficiente, ceea ce pune viețile pacienților în pericol și limitează opțiunile terapeutice disponibile. În fața acestei crize globale, cercetările recente deschid noi direcții în combaterea antibiorezistenței, concentrându-se pe descoperirea de antibiotice inovative, soluții alternative și strategii de prevenire a răspândirii rezistenței. Aceste progrese sunt esențiale pentru a răspunde provocărilor viitoare în domeniul sănătății.

Abstract

Antibiotics have transformed medicine, enabling effective treatments for infections that were once fatal and supporting significant advancements such as surgery and organ transplants. However, the growing spread of resistance reduces their effectiveness, making the discovery of new drugs crucial in the ongoing battle against human pathogens. Antibiotic resistance represents one of the greatest challenges of modern medicine, having a significant impact on the treatment of bacterial infections. The development of antibiotic resistance renders standard treatments ineffective, putting patients' lives at risk and limiting available therapeutic options. In the face of this global crisis, recent research is opening new directions in combating antibiotic resistance, focusing on the discovery of innovative antibiotics, alternative solutions, and strategies to prevent the spread of resistance. These advances are essential for addressing future health challenges.

1. The discovery of a new antibiotic in soil microbes from North Carolina.

A new antibiotic, produced by bacteria grown in the laboratory, can destroy "superbugs" without causing them to become more resistant to treatment, according to a preliminary study.

Researchers isolated the antibiotic, named clovibactin, from a bacterium called *Eleftheria terrae* subspecies *carolina*, which they collected from soil samples in North Carolina [20].

Clovibactin is a novel chemical antibiotic derived from what is referred to as the "dark matter" of bacteria—bacteria that had not been previously cultivated in the laboratory.

It is non-toxic, as evidenced by animal experiments, and proves to be more effective than the reference antibiotic vancomycin, which is used to treat bacterial infections resistant to other drugs but is beginning to

lose efficacy against some bacterial strains [19].

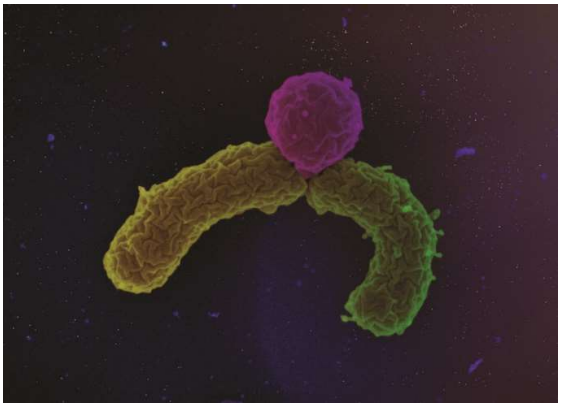

Fig. 1. *Eleftheria terrae* subspecies *carolina*.
Source: William Fowle, Northeastern University [42]

The traditional mechanisms of antibiotic action

Antibiotics act through various mechanisms to destroy or inhibit bacterial growth, targeting the unique characteristics of bacterial cells and how they differ from human cells.

The main mechanisms of antibiotic action include:

Inhibition of cell wall synthesis:

The bacterial cell wall is essential for their integrity. Some antibiotics, such as penicillins and cephalosporins, prevent the synthesis of this wall, leading to the rupture of bacteria.

They bind to the enzymes involved in cell wall formation, resulting in bacterial cell lysis and the death of the bacterium.

Inhibition of protein synthesis:

Bacteria have their own ribosomes, which differ from those in human cells, and antibiotics targeting protein synthesis inhibit bacterial ribosome activity. For example, macrolides (such as erythromycin) and tetracyclines bind to bacterial ribosomes, blocking the process of translating genetic information into proteins, a critical step for bacterial survival [9].

Inhibition of nucleic acid synthesis:

Other antibiotics, such as quinolones and rifamycins, inhibit bacterial enzymes involved in DNA and RNA synthesis.

For example, quinolones block DNA gyrase, an enzyme crucial for bacterial DNA replication. Without this replication mechanism, bacteria cannot multiply and die.

Alteration of cell membrane permeability:

Certain antibiotics, such as polymyxins, disrupt the structure of bacterial cell membranes, causing them to lose integrity.

These antibiotics form channels in the membrane, allowing the leakage of essential cellular contents, which ultimately leads to bacterial death [41].

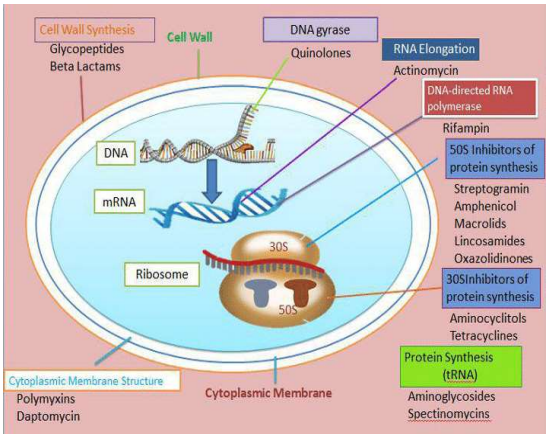

Fig. 2. Different mechanisms of antibiotic action
Source: https://www.researchgate.net/figure/The-mechanism-of-action-of-different-types-of-antibiotics-Antibacterial-action-generally_fig4_315925913 [43]

Inhibition of bacterial metabolism:

Some antibiotics, such as sulfonamides, interfere with bacterial metabolism by inhibiting enzymes involved in the synthesis of folic acid, a substance essential for bacterial replication.

These antibiotics act by blocking a key step in metabolism, preventing bacteria from producing the materials needed for growth [37].

Action on lipid biosynthesis:

Some antibiotics, such as lipopeptides, target lipid biosynthesis in the bacterial membrane.

They disrupt the formation and maintenance of the bacterial membrane, ultimately leading to bacterial cell death.

Each of these mechanisms targets a component or process essential for bacterial survival.

Depending on how the antibiotic acts, it can be classified as bactericidal (destroying bacteria) or bacteriostatic (inhibiting bacterial growth and reproduction).

Additionally, antibiotics can be broad-spectrum, affecting a large number of bacterial types, or narrow-spectrum, targeting only specific bacterial strains. [29].

Uncultivated bacteria

Uncultivated bacteria represent a vast (nearly 99% of all species) and untapped source of new natural product structures.

Recently, the development of the iChip technology has provided access to a wide diversity of uncultivated bacterial species, leading to the discovery of teixobactin, which was isolated from the soil bacterium *Eleftheria terrae* [26].

Teixobactin exhibits excellent antibacterial activity and a unique chemical structure. It blocks cell wall biosynthesis by specifically binding to lipid precursors, forming supramolecular structures that disrupt membrane stability [33].

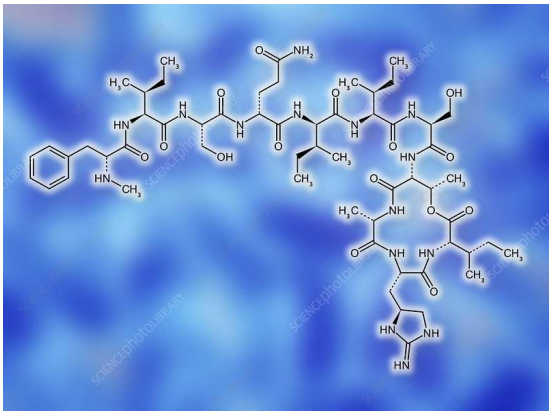

Fig. 3. Teixobactina – chemical formula
Source:https://www.sciencephoto.com/media/642105/view/teixobactin-antibiotic-formula [44]

Other antibiotics with novel mechanisms of action discovered through screening uncultivated bacteria include lassomycin, an inhibitor of the ClpP1P2C1 protease, and amycobactin, an inhibitor of the SecY protein export system, both of which act selectively against mycobacteria.

Therefore, uncultivated bacteria appear to provide a rich source of compounds with unique chemical and mechanistic properties, which is promising for the continued discovery of effective antibiotics and the development of next-generation therapies.

In this context, we report the discovery and mode of action of clovibactin, an antibiotic with no detectable resistance, identified through the screening of uncultivated bacteria [35].

The discovery of clovibactin

Previously, it was difficult to cultivate *E. terrae* subspecies *carolina*, as it requires specific nutrients and symbiotic microbes from the soil in which it naturally grows.

However, researchers have developed a device that can recreate the necessary conditions to cultivate this rare microbe in its natural soil environment, he added. [40].

Clovibactin is an innovative, recently discovered peptide antibiotic with a unique chemical structure and a promising mechanism of action in combating antibiotic-resistant bacteria. It is produced by a bacterium found in soil in North Carolina (*Eleftheria terrae*), a microbe that had not been previously cultivated in the laboratory [5].

The structure of clovibactin includes a linear sequence of amino acids, notably featuring two D-amino acids and a rare residue, D-3-hydroxy-asparagine, within a depsipeptide cycle.

These unusual chemical characteristics provide the compound with stability and effectiveness in combating bacteria.

Although its structure is similar to that of another antibiotic, teixobactin, clovibactin has

a shorter linear N-terminal end and differs in the specific arrangement of its residues, making it unique among other peptide antibiotics [30].

A remarkable aspect of clovibactin is that no significant resistance has been identified so far, suggesting high potential in combating pathogenic bacteria, including multidrug-resistant strains.

This antibiotic acts by inhibiting bacterial cell wall biosynthesis, a process crucial for bacterial growth and survival. Clovibactin binds to highly conserved lipid precursors, disrupting the bacterial membrane structure and preventing their replication.

Due to these characteristics, clovibactin represents a potential solution in the fight against antibiotic-resistant infections and opens new possibilities for the development of more effective drugs in treating bacterial diseases. [32].

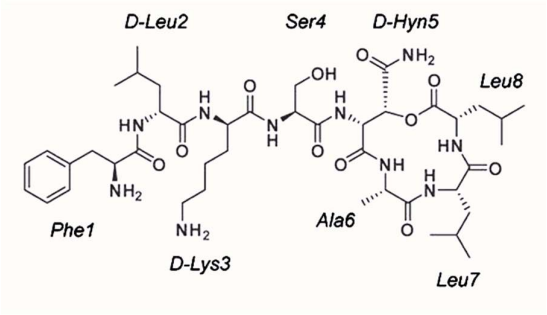

Fig. 4. Clovibactin – chemical formula
Source: https://www.the-chemistry-editor.com/post/unearthing-clovibactin-a-breakthrough-depsipeptide-antibiotic-defying-drug-resistance [45]

The efficacy of clovibactin

Clovibactin has demonstrated antibacterial activity against a broad spectrum of Gram-positive pathogens, including strains of *S. aureus* resistant to methicillin (MRSA), resistant to daptomycin, and with intermediate resistance to vancomycin (VISA), as well as against difficult-to-treat strains of vancomycin-resistant *Enterococcus faecalis* and *E. faecium* (VRE).

In contrast, *Escherichia coli* was only marginally affected, compared to the *E. coli* WO153 strain, which had defects in its outer membrane, suggesting insufficient penetration of the compound in this case.. [39].

It has been discovered that clovibactin can destroy two dangerous superbugs: methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecalis*. MRSA can cause life-threatening infections when it enters the body through wounds or during surgical procedures, while *E. faecalis* leads to various types of infections, including urinary tract infections. [13].

Clovibactin is a bactericidal antibiotic, with a minimum bactericidal concentration (MBC) for *S. aureus* being twice the minimum inhibitory concentration (MIC).

Researchers studied its time-dependent effect on bacterial elimination and found that clovibactin is more effective in killing *S. aureus* than vancomycin, a frontline antibiotic. They observed a strong lysis of bacterial cultures and quantified this effect.

In the past, teixobactin was shown to be rapidly bactericidal, inducing bacterial lysis mediated by the AtlA enzyme, a major cell wall autolysin in *S. aureus*. [31].

The pronounced lysis observed with clovibactin is typical of compounds that act as detergents, rapidly disrupting the cell membrane. However, the lysis induced by clovibactin does not result from the rapid formation of pores or membrane dysfunction, as evidenced by the absence of effects on the membrane in permeability tests, unlike the lantibiotic nisin. [17].

Treatment with clovibactin did not result in the rapid depolarization of the membrane, unlike the action of teixobactin, which weakens and depolarizes bacterial membranes [34].

The mechanism of action of clovibactin

The inability of *S. aureus* bacteria to resist clovibactin may be due to the unique

way this antibiotic destroys them: it targets undecaprenyl pyrophosphate, a chemical group found in three lipid molecules that form the building blocks of the bacterial cell wall.

Clovibactin surrounds these molecules like a cage, which is why its name is derived from the Greek word "klouvi," meaning "cage" [36].

Since bacteria cannot easily modify these building blocks without compromising the cell wall, it would be very difficult for them to develop resistance to clovibactin.

Clovibactin binds to the pyrophosphate (PPi) group of several essential cell wall precursors.

Typically, PPi is not considered a suitable target for an antibiotic, as it is released from dead cells along with phosphorylated nucleosides containing PPi [15].

However, pyrophosphate is both an essential component and an immutable part of the cell wall lipid intermediates, unlike other parts of the molecules, such as the pentapeptide in lipid II or sugars, which can be modified (for example, in *mycobacteria*) or altered through mutations [25].

Moreover, the substitution of D-Ala-D-Lac in the lipid II pentapeptide is a common mechanism of resistance to vancomycin.

Binding to the immutable PPi group would explain the absence of detectable resistance to this compound.

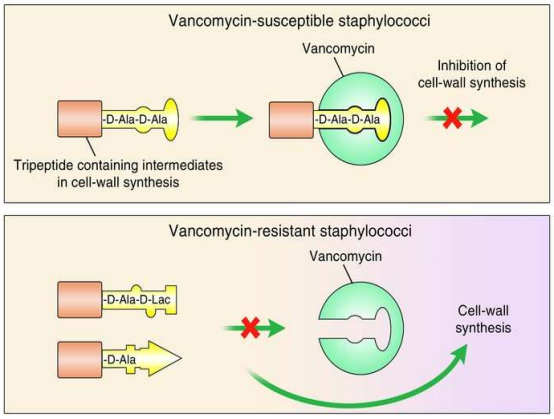

Fig. 5. Vancomycin resistance
Sursa: onlineacademiccommunity.uvic.ca [46].

Clovibactin toxicity

Clovibactin showed no cytotoxic effects on mammalian cells (NIH/3T3 and HepG2) at a concentration of 100 µg/mL (the highest concentration tested).

Given its strong antimicrobial activity and low cytotoxicity, the compound was further evaluated in in vivo studies. In a pharmacokinetic (PK) study, to assess systemic exposure and blood retention time, a single intravenous dose of clovibactin was administered to mice at doses of up to 40 mg/kg, and it was well tolerated. [11].

Intravenous injections of clovibactin were also more effective than intravenous vancomycin in reducing *S. aureus* levels in infected mice.

Although no side effects were observed in mice, further studies in humans are necessary to confirm the antibiotic's efficacy and assess its safety. [23].

2. New methods for identifying antibiotics using AI technology

Artificial intelligence could identify the next drug in the fight against superbugs.

To create new antibiotics, researchers typically locate the genes that confer resistance to bacteria.

Through laboratory experiments, they analyze how bacteria respond to various antibiotics and search for mutations in their genetic structure that enable them to resist treatment.

Although this approach is effective, it can be time-consuming and does not always capture the full complexity of how bacteria become resistant.

For example, changes in gene function, even without mutations, can influence resistance.

Additionally, bacteria can transfer resistance genes between each other, a phenomenon that may be overlooked when focusing solely on mutations within a single strain [1]

Identification of antibiotic resistance

To identify genes involved in resistance, researchers studied the genomes of different strains of *E. coli*, searching for patterns and genetic markers associated with resistance.

Machine learning algorithms, trained on existing data, were then applied to discover new genes or common mutations in resistant strains that could promote the development of resistance [3].

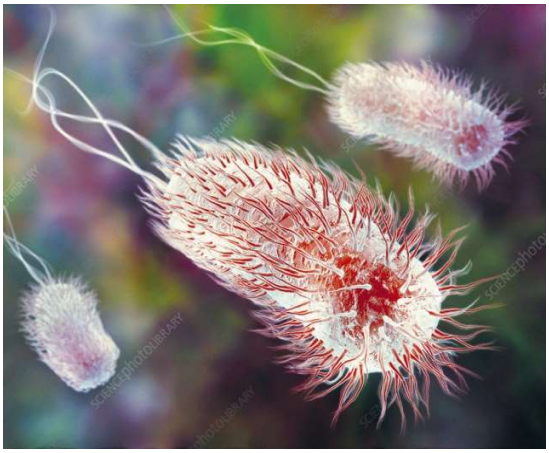

Fig. 6. *E. coli*
Source: Hybrid medical animation / science photo library [47]

After identifying resistance genes, researchers designed inhibitors that specifically target and block the proteins produced by these genes.

By analyzing the structure of the proteins encoded by these genes, they managed to optimize the inhibitors to bind strongly and specifically to these proteins [2].

E. coli is a Gram-negative bacterium widely present in the environment and commonly found in the gastrointestinal tract of humans and warm-blooded animals.

While most strains are harmless, certain pathogenic variants have the potential to cause various diseases, ranging from relatively simple urinary tract infections to severe bloodstream infections.

The urgent need for new therapeutic compounds is highlighted by the rise of multidrug-resistant (MDR) bacteria, which are resistant to multiple classes of antibiotics.

Therefore, a comprehensive study of the genetic basis of antibiotic resistance in *E. coli* is essential for advancing antimicrobial drug development. [38].

Sequencing Techniques

The use of whole-genome sequencing (WGS) has significantly advanced the process of decoding the genetic structures of bacterial pathogens, enabling a detailed and precise examination of their genomes.

By clarifying the genetic factors that contribute to antibiotic resistance, WGS allows for the identification of potential target genes that can be suppressed.

Additionally, comparative genomics facilitates the investigation of evolutionary links between resistant and susceptible strains, providing insights into the mechanisms behind the acquisition and spread of resistance [12].

Genome annotations are labels assigned to different sequences in the bacterial genome, indicating the potential functions of genes, regions of interest (such as resistance genes, essential enzymes, etc.), and interactions between various genomic elements. This aids in understanding the role of each part of the genome in the bacterium's biological processes. [21].

Subsystems refer to functional groups of genes that are involved in specific biological functions or processes. These subsystems include, for example, processes such as metabolism, protein synthesis, DNA repair, and more.

Analyzing subsystems helps identify metabolic pathways and biological processes that are crucial for the survival and virulence of bacteria. [7].

The goal of this technique is to use predictive modeling and computational methodologies to identify potential genes involved in resistance mechanisms within the E. coli genome.

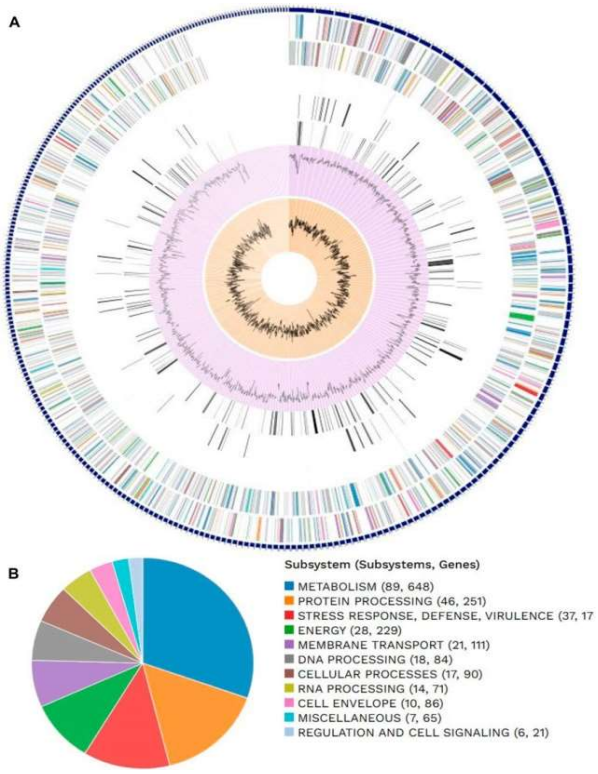

Fig. 7. Distributions (A) of genome annotations and (B) of subsystems in *Escherichia coli.*
Source: https://www.frontiersin.org/journals/bioinformatics/articles/10.3389/fbinf.2024.1411935/full [48]

Furthermore, *in silico* screening methods, such as molecular docking, molecular dynamics simulations (MD), and ADMET profiling, are employed to develop small-molecule inhibitors that specifically target these potential genes.

The ultimate aim of this approach is to restore the effectiveness of antibiotic therapy [8].

The in silico screening methodology refers to the use of computational technologies and simulations to analyze and evaluate the biological potential of chemical substances or molecules before experimental testing.

"In silico" comes from the Latin term meaning "in silicon," referring to the use of computers and data-driven simulations to study biological, chemical, or pharmacological processes [6].

In the context of discovering new drugs or developing treatments, the in silico screening methodology includes multiple techniques and methods:

1. Molecular Docking: This technique involves simulating the interaction between a molecule (usually a potential active compound) and a biological target, such as a protein or receptor. The goal is to predict how the molecule will bind to its target and to assess its potential to inhibit or modify its activity [10].
2. Molecular Dynamics (MD) Simulations: These simulations allow the study of molecular behavior over time, providing insights into how molecules move and interact in a simulated environment. In the context of drug research, MD helps in understanding interactions between drugs and target proteins. [14].
3. ADMET Profiling: ADMET refers to four key properties of a chemical substance: absorption, distribution, metabolism, excretion, and toxicity. ADMET profiling predicts how a substance will be

absorbed, distributed, metabolized, and excreted in the body, as well as whether it may have toxic effects. This helps in selecting promising chemical compounds and improving their safety before clinical testing [16].

These methods save time and resources by reducing the need for extensive experimental testing, allowing researchers to quickly identify promising candidates for the development of new drugs or treatments. Additionally, they can be used to gain a better understanding of the molecular mechanisms of diseases and to design more effective therapies.

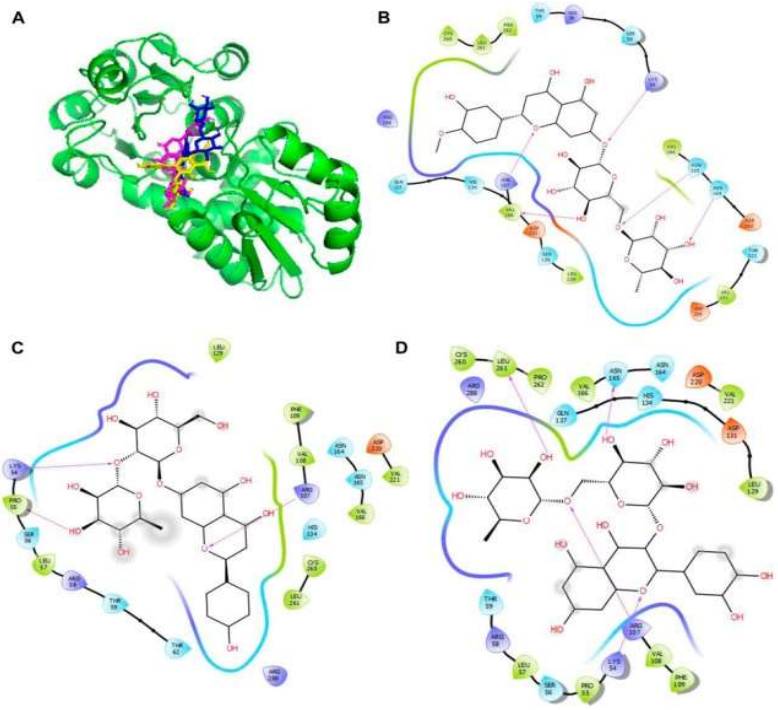

Fig. 8. Molecular docking of bioactive compounds in KatG from E. coli. (A) The 3D binding positions of KatG (green), hesperidin (blue), naringin (purple), and rutin (yellow) in the binding cavities of KatG from E. coli. The 2D interaction analyses of (B) hesperidin, (C) naringin, and (D) rutin with amino acid residues in the binding cavities of KatG from *E. coli* **Source:** https://www.frontiersin.org/journals/bioinformatics/articles/10.3389/fbinf.2024.1411935/full [49]

5. Conclusions

In conclusion, in the face of increasing challenges posed by antibiotic resistance, new research directions focus on identifying novel therapeutic targets and developing innovative antibiotics that act through mechanisms distinct from traditional treatments. Approaches such as genome sequencing technologies, molecular modeling, and in silico screening have opened new opportunities for discovering compounds with promising antimicrobial activity capable of combating resistant strains. Additionally, studies on hard-to-culture bacteria and the exploration of previously unknown mechanisms, such as the inhibition of essential cell wall synthesis precursors, have made significant advances.

These developments, along with research into the safety and efficacy of new compounds, pave the way for the creation of more effective and sustainable treatments, ultimately ensuring long-term protection of public health.

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