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AI-Powered De Novo Antibiotics Discovery: Is It the Answer to Overcome Antimicrobial Resistance? A Systematic Review of Preclinical Evidence Across *In Vitro* and *In Vivo* Studies

Abstract

Introduction: Antimicrobial resistance (AMR) remains a critical global issue. By 2050, it is projected to cause around 10 million deaths if current trends persist. Traditional antimicrobial discovery struggles to keep up with rapidly evolving resistance due to its lengthy process, high cost, and high failure rate. Developing a single drug can take over a decade of research and cost millions of dollars. These challenges demand more efficient approaches, with artificial intelligence (AI) offering a promising path to accelerate and improve antibiotic development.
Methods: GoogleScholar, PubMed, ScienceDirect, and Scopus were systematically searched following the PRISMA 2020, yielding 13 eligible studies. All included *in vitro* validation, and four extended to *in vivo* investigations. Risk of bias was evaluated using the QUIN (*in vitro*) and the SYRCLE (*in vivo*) tools.
Discussion: Across studies, AI supported multiple stages of antibiotic discovery, including target identification, lead compound optimization, also enhancement of pre-clinical testing. In target identification, two studies revealed novel antibacterial targets distinct from classical pathways. During lead optimization, applied in most studies, AI-generated compounds demonstrated strong antimicrobial activity and low MIC values against broad-spectrum and multi-drug resistant bacteria. Four *in vivo* studies further showed that these de novo antibiotics exhibited superior antimicrobial efficacy to current standard therapies. Finally, in preclinical testing, AI models accurately predicted cytotoxicity and hemolysis, later confirmed experimentally.
Conclusion: AI has markedly improved efficiency and accuracy in antibiotic development. While continued model refinement, validation, and ethical oversight remain crucial, AI-integrated pharmaceutical research indicates growing maturity and transformative potential.
Keywords: artificial intelligence, antibiotic discovery, antimicrobial resistance, preclinical evidence

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1. INTRODUCTION

Antimicrobial resistance (AMR) remains a critical global health challenge, responsible for substantial mortality worldwide, particularly in developing countries. In 2021 alone, bacterial AMR was associated with an estimated 4.71 million deaths.[1] While the global median rate of antibiotic resistance reached 17.2%, the Southeast Asian region, especially developing countries such as Indonesia, recorded significantly higher levels, with resistance rate averaging 31.1%.[1] Given its profound health and economic impact, investing in AMR containment has been identified as one of the highest-yield public health investments countries can make.[2] Global modelling estimates that, under a better care scenario, which includes expanded access to effective antibiotics, an estimated 92.0 million deaths could be averted within two decades.[3]

A key driver of antibiotic failure is the natural selection of resistant bacterial phenotypes.[2-3] Traditional drug discovery is a lengthy and resource-intensive process, often requiring up to 15 years and costing up to US $2.8 billion per drug on average.[4] Much of this burden roots from high failure rate in clinical development, with nearly 90% of candidates ultimately failing even after successfully passing Phase I clinical trials.[4-5] In addition, commonly used methods such as high-throughput screening (HTS) yield only about a 2.5% hit rate, further prolongs development timelines and increases costs.[6]

Given these limitations, innovative approaches such as artificial intelligence (AI)-assisted drug discovery have begun to transform the development landscape.[7] Notably, recent development of an obsessive-compulsive disorder drug, DSP-1181, became the first AI-designed drug to enter a phase I clinical trial. It required less than 12 months from initial screening to preclinical testing, compared with roughly four years typically required using traditional drug-development pipelines.[6] The progress achieved through AI in other therapeutic areas highlights the urgent need to apply similar strategies in antibiotic development. Despite the growing threat of antimicrobial resistance, only five new antibacterial agents have been developed in the past two decades, reflecting a concerning stagnation in the antibiotic pipeline.[8] Consequently, AI approaches are now widely used to screen the activity of chemical compounds against various pathogens including bacteria.[8-9]

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AI-assisted drug discovery relies on computational frameworks that accelerate key steps in development.[9-11] Machine learning enables rapid target identification, deep-learning models generate and screen novel compounds in silico, and predictive algorithms assess ADMET properties early, while structured-based tools support efficient lead optimization.[10] Together, these approaches shorten timelines, reduce costs, and improve success rates, making them especially valuable for revitalizing antibiotic discovery.[9-11] Accordingly, this study aims to evaluate the role of AI in antibiotic discovery, with a particular focus on preclinical *in vitro* and *in vivo* evidence.

2. METHODS

Search Strategy

Three reviewers performed the literature search following the Preferred Systematic Reviews and Meta Analyses Statement (PRISMA) guidelines, using GoogleScholar, Pubmed, ScienceDirect, and Scopus as databases.[12] The keyword strategy for this review was developed based on three main domains: AI, antibiotics studies including antimicrobial peptides (AMPs), and in vitro and *in vivo* studies. All search terms were systematically derived, connected through boolean operators, and listed in detail in **Supplementary Table 1.**

Inclusion and Exclusion Criteria

Studies included in this review are those which fulfil the criteria; use AI at any stage of drug development, particularly for compound optimization, clear description of the AI methods or models applied, had progressed to either *in vitro* or *in vivo* testing, and publication within the last 10 years.

The exclusion criteria for this review were non-English or non-Indonesian articles, studies that were not accessible in full text, purely in silico research without experimental validation, and studies in which the identified compounds were not de novo but involved drug repurposing.

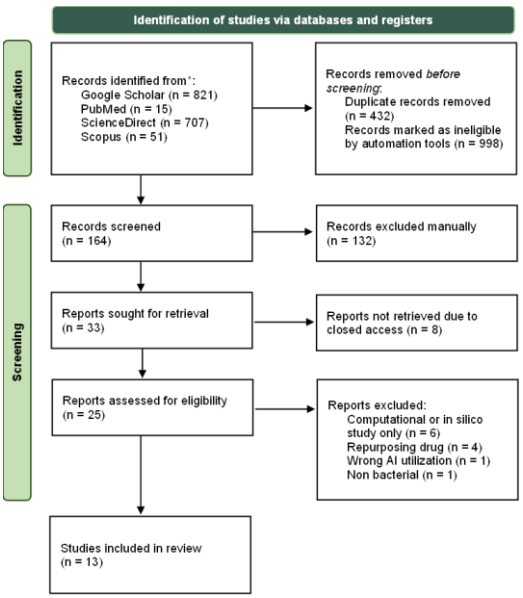

Figure 1. Study selection flow following PRISMA Guideline1[12]

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Study Selection

After searching the specified databases using filters and several automation tools, 164 articles were collected. Following exclusion criteria, 25 studies were reviewed in full text. In total 13 studies were found eligible for this review. The PRISMA flow chart corresponding to the present methodology is shown in **Fig 1**.

Quality Assessment

Risk of bias was assessed using the Quality Assessment Tool for *In Vitro* Studies (QUIN) tool for *in Vitro* studies and the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE)'S risk of bias tool *for in vivo* studies. All of the included studies show low risk bias both in both *in vivo* and *in vitro*, except one study showing moderate risk. Detailed bias score distributions for each study are presented in **Supplementary Table 2 & Supplementary Table 3**, with a summarized representation shown in **Fig 2 & Fig 3**.

Included *in vitro* studies assessed with QUIN tools were all marked as not applicable (NA) in several domains. Specifically in sample size, randomization, outcome assessor, and blinding. This is due to the objective of the included *in vitro* studies were using standardized laboratory procedures applied uniformly to bacterial cultures or cell lines, rather than assigning interventions to individual subjects. Outcomes such as minimal inhibitory concentration (MIC) values, optical density (OD) measurements, or colony forming units (CFU) counts are generated through objective, instrument-based readouts, which are not influenced by assessor judgment. For this reason, such domains are not typically relevant in controlled *in vitro* settings. Marking these items as NA reflects methodological appropriateness, not missing safeguards.

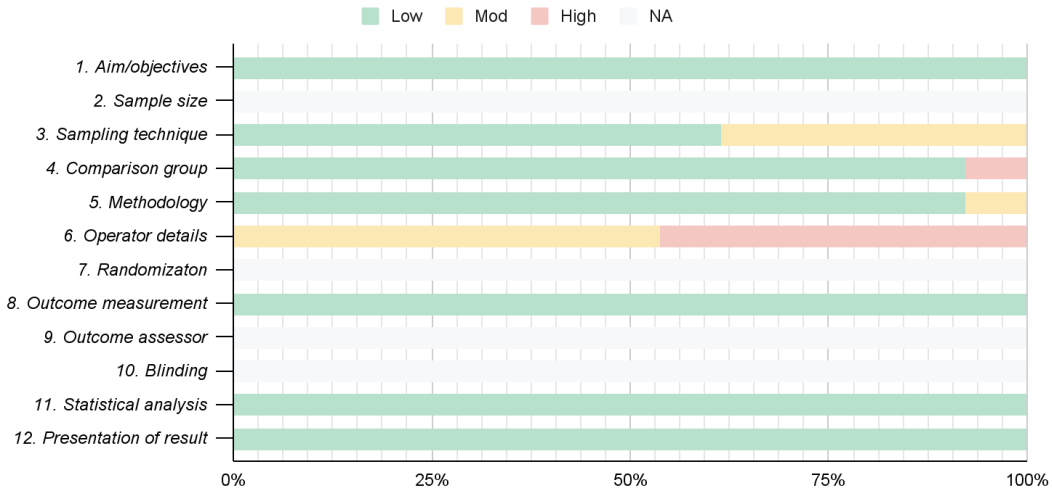

Figure 2. Distribution of risk-of-bias scores assessed using the QUIN tool [13]

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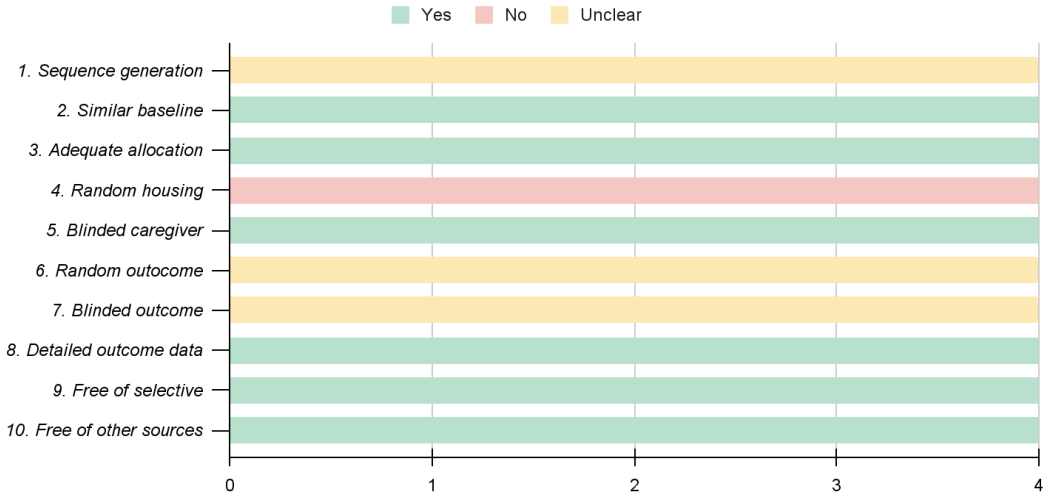

Figure 3. Distribution of risk-of-bias scores assessed using the SYRCLE's RoB tool [14]

Lastly, as *in vivo* experiments in these studies primarily designed as confirmatory validation of antimicrobial efficacy observed *in vitro*, most did not report details on several methodological domains, including sequence generation, random housing, random outcome, and blinded outcome assessment. Despite this, they are unlikely to have introduced significant bias in the interpretation of outcomes.

3. RESULT & DISCUSSION

Study Characteristics

This systematic review identified 13 eligible studies applying AI for de novo antibiotic discovery. All included investigations conducted *in vitro* experimental validation, while four studies further extended their evaluation into *in vivo* preclinical models. Across the dataset, 4 studies focused on small-molecule antibiotic design, and the remaining 9 studies centered on AMP discovery. Detailed AI methodological characteristics, validation strategies, and target organisms for each study are summarized in **Fig 4** and **Supplementary Table 4**.

Generative AI Models: Theoretical Architecture

Generative AI models have become a foundational component of modern antibiotic discovery.[9] Across the studies reviewed, frameworks such as variational autoencoders (VAEs), diffusion models (DMs), generative adversarial networks (GANs), transformer-based, and neural network models (NNMs) - based models constitute the core computational approaches used in AI-assisted antibiotic design. These models learn mappings between molecular or peptide descriptors and biological activity or other relevant chemical properties.[15] These principal

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architectures used in this domain are illustrated in **Fig 4a**.

1. *Neural network models (NNMs)*
   Earliest developed artificial intelligence models were neural networks such as recurrent neural networks (RNNs), convolutional neural networks (CNNs), graph neural networks (GNNs), and deep neural networks (DNNs).[10,16] These models learn statistical patterns in biological or chemical data and are widely used as predictive tools to classify antimicrobial activity, estimate MIC values, predict toxicity, or assist in filtering AI-generated candidates.[9]

2. *Variational autoencoders (VAEs)*
   A VAE is a type of generative model that learns to create new data by learning a probabilistic representation of the input data's latent space.[9-10,16] VAE learns to compress sequences or molecules into a continuous latent space and then reconstruct them back into valid peptide or chemical structure.[18] The key theoretical strength of VAEs lies in their ability to transform discrete biological sequences (amino acids sequences or molecular trings (SMILES)) into a smooth, navigable latent landscape, allowing controlled interpolation, mutation, and optimization.[18-19]

3. *Generative adversarial networks (GANs)*
   GANs rely on a competitive framework between two neral networks, a generator and a discriminator.[10,16,20] The generator proposes new sequences or molecules from random noise and the discriminator evaluates whether these sequences resemble realistic antimicrobial properties.[10] GANs excel at capturing complex, high-level patterns found in AMP datasets, although they may suffer from training instabilities or mode collapse

4. *Diffusion models (DMs)*
   Diffusion models represent a newer class of generative AI inspired by nonequilibrium thermodynamics.[9,16,22] They operate by incrementally adding noise to training sequences and then create new data by learning to reverse a gradual noising process (denoising).[9,21] These features allow DMs to generate novel structures not restricted by template similarity and exploration of highly diverse chemical or peptide spaces.[22-23]

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Figure 4. Overview of AI Frameworks and Study Distributions.
(A) Schematic illustration of the major deep generative model frameworks applied, including variational autoencoders (VAEs), generative adversarial networks (GANs), diffusion models (DMs), and transformer-based architectures. (B) Distribution of included studies according to the type of AI model utilized (C) Distribution of target bacterial species investigated.

5. *Transformer-based models*
Transformers are neural networks built around self-attention rather than recurrence (RNNs) or convolution (CNNs).[9] Their core mechanism allows the model to evaluate relationships among all positions in a sequence

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simultaneously, capturing complex dependencies with high fidelity.[9,24] When applied to small molecule strings (SMILES or SELFIES) and protein sequences, transformers treat chemistry as a language.[9,24] In doing so, they provide a framework that links sequence information with structural and functional properties.[24-25] Transformer-based approaches leverage large unlabeled datasets through self-supervised learning, enabling the derivation of latent representations that are broadly useful for tasks such as property prediction, structure generation, and analog design.[26-27] These capabilities support the generation of both protein and molecular structures by capturing complex sequence dependencies through attention-based encoding.[27]

NNMs, VAEs, GANs, DMs, and transformers architectures each provide a distinct methodological perspective for learning and sampling chemical space.[9] Hybrid architectures, such as diffusion models incorporating transformer components or VAEs augmented with discriminator modules inspired by GANs, are increasingly common.[17] These integrated approaches are designed to leverage complementary strengths while mitigating the limitations inherent to individual model types.[9,17]

Previously described generative frameworks operate over a range of molecular representations that encode chemical information in different ways.[9] String-based or linear formats such as SMILES and SELFIES capture molecular graphs as linear tokens suitable for language-model architectures; graph-based representations provide explicit atom-bond connectivity for models that reason over molecular topology; and three-dimensional coordinate-based formats capture spatial geometry essential for structure-aware generation.[28] Peptide and protein sequences are typically represented as amino-acid strings, enabling natural-language–style modeling, while emerging representations integrate sequence, structural constraints, and physicochemical annotations.[28-29]

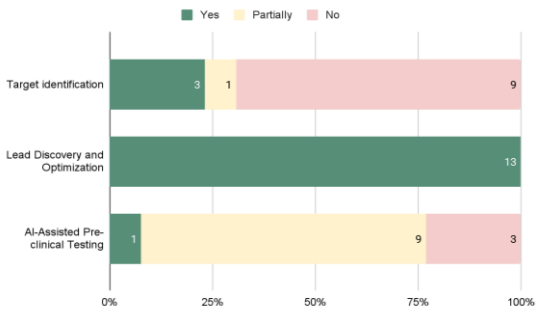

Figure 5. Application of AI across the antibiotic discovery pipeline

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Taken together, these core architecture of AI models form the foundation of modern AI-driven antibiotic discovery. With this computational backbone established, the next section explores how these AI systems are applied in each step of molecular generation, starting with identifying novel antibacterial targets to guide early-stage discovery. A summary of how AI contributed across key discovery steps for each included study is presented in **Fig 5** and **Supplementary Table 5.**

AI-assisted Target Identification

Target identification is a foundational step in antibiotic discovery, but traditionally it requires labor-intensive genomic analysis, biochemical screening, or comparative evolutionary studies. AI algorithms have revolutionized this phase by efficiently processing vast amounts of biological data from multiple sources.[20] AI assisted the early stage of discovery by analyzing large-scale genomic, proteomic, metagenomic, and chemical-biological interaction data to reveal druggable targets and previously unexplored antimicrobial mechanisms.[30-31] For target identification, diverse AI model architectures can be applied, depending on the data source and desired output.[20,31] Among four studies investigating potential druggable targets for newly generated antibiotics, two studies stand out for their notable findings.

The first one is a study that employed GNN models to screen large, enumerated chemical fragment spaces, scoring each fragment according to its predicted antibacterial properties.[8] Through this approach, a fragment that targets LptA in gram-negative bacteria, especially *Neisseria gonorrhoeae* and *Staphylococcus aureus*, were identified as one of the most potent candidates.[8] To date, no FDA-approved antibiotic shares this mechanism of action.[8,32] LptA is a component of the lipooligosaccharide (LOS) export system, responsible for transporting lipopolysaccharide (LPS) molecules from their site of assembly in the inner membrane, across the aqueous periplasm, to their final cell surface location during outer membrane biogenesis.[8,34] Through a series of AI framework refinements, this fragment was further optimized into a newly generated antibiotic molecule, demonstrating the ability of AI-driven approaches to substantially reduce the effort required to identify and validate highly effective druggable targets specifically for small molecule types of antibiotics.[8]

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Unlike many small-molecule antibiotics that begin by identifying a specific molecular target, de novo AMP studies predominantly optimize toward an overall antimicrobial phenotype rather than a single protein or defined cellular pathway.[35] What is striking across the included studies is that, although membrane disruption is the shared biological endpoint, the AI systems do not converge on a single structural formula for achieving it. Instead, they uncover multiple, distinct physicochemical solutions that all lead to effective membrane-targeting activity.[36]

This is best illustrated in the ApexAmphion study by Cao et al where AI models, rather than producing one dominant structural motif, explored a wide variety of sequence architectures that each enabled membrane disruption in different ways.[36] Some AI-designed peptides achieved activity through strong cationicity, enhancing electrostatic attraction and initial binding to the bacterial surface.[36] Others relied on optimized amphipathic helices capable of inserting deeply into lipid bilayers to initiate pore formation. Another subset favored hydrophobic or aromatic clustering, promoting bilayer penetration through entirely different structural dynamics. The AI even generated shorter, rigorously patterned motifs that destabilized membranes without relying on classical helical structures. Despite their differences, all of these designs ultimately converged on the same antimicrobial effect, compromising membrane integrity and inducing rapid bacterial killing.[36]

Another significant application of AI-assisted target validation is its ability to dissect and refine the mechanistic landscape of nitrofuran-based antibiotics, a class with existing FDA-market representatives but still only partially understood modes of action.[37] AI frameworks did not merely replicate known nitrofuran scaffolds. Instead, it systematically explored structural improvisations to enhance antibacterial potency and refine mechanistic performance.[38] Most notably, the substitution of the traditional hydrazone linker found in marketed nitrofuran antibiotics with an acrylamide moiety.[38] This structural shift significantly altered redox behavior, enabling modified activation pathways and potentially hybrid mechanisms of action beyond the parent class.[39] Rather than discovering an entirely new biological target, this work underscores AI's unique ability to optimize and reinvent existing mechanisms, revealing which structural perturbations yield the most efficient antibacterial responses.[38]

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Although only a subset of studies in this review directly applied AI for target identification, these works demonstrate the emerging potential of machine learning to expand the antibiotic target landscape. As generative and predictive models become increasingly sophisticated, AI is not only capable of uncovering previously overlooked biological vulnerabilities but also of redefining how we conceptualize antimicrobial targets altogether.

Generative-AI for Lead Discovery, Optimization, and Hit Identification

Across all included studies, generative AI frameworks operate by learning the underlying statistical, structural, and physicochemical patterns that define antimicrobial activity.[8,36] These datasets span curated AMP and small molecule libraries of only a few thousand sequences to ultra-large small-molecule collections that exceed one billion compounds[40-41], giving models access to chemical and biological spaces far beyond what is tractable by manual design or conventional screening[4].

By embedding necessary parameters into their optimization process, AI models can forecast biological behavior that typically requires extensive laboratory testing far earlier in the discovery pipeline[36,40-41]. Furthermore, the scale of AI-based generative exploration greatly exceeds what is feasible experimentally. Several studies generated hundreds of thousands to millions of virtual peptides or chemical structures, systematically narrowed them through AI predictors, and then progressed only the top-scoring candidates to synthesis[8,36]. The complete summary of dataset sizes, number of generated compounds, synthesized molecules, and number of experimentally validated hits for each study is provided in **Supplementary Table 4**.

AI-generated antibiotics were primarily evaluated against pathogens within the ESKAPEE group (*Escherichia coli, S. aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp*), reflecting the global clinical priority to address these highly drug-resistant organisms.[42] The most frequently targeted species were *E. coli* (24%) and *S. aureus* (24%), including their resistant phenotypes such as methicillin-resistant *S. aureus* (MRSA)[21] and ciprofloxacin-resistant *S. aureus*[43]. This focus aligns with their high prevalence, rising resistance rates, and their role as major contributors to hospital-acquired infections[42]. Several studies also extended their evaluation to other high-concern

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pathogens, including *N. gonorrhoeae*[8], *Enterococcus faecium,*[36] *Proteus mirabilis,*[40] *Salmonella enterica,*[36] and many more, further demonstrating the broad applicability and therapeutic relevance of AI-designed candidates.

In vitro validation across studies predominantly relied on MIC assays to quantify antibacterial potency, often benchmarked against standard antibiotics such as vancomycin[8], polymyxin B[43], ciprofloxacin[5], or reference AMPs. MIC determination was complemented by additional functional assays in several investigations, including CFU reduction[8], membrane permeabilization assays, time-kill curves, biofilm inhibition and eradication assays, cytotoxicity and hemolysis profiling, and molecular interaction characterizations such as redox activation or membrane-binding dynamics.[5,40,41-42] Distribution of assays that were conducted from each *in vitro* studies were provided in **Fig 6**. These complementary assays strengthened the interpretation of antimicrobial efficacy and provided mechanistic insights into how AI-generated molecules exert their activity.

Across all studies, the top hits generated by AI demonstrated clear antimicrobial activity, including potent effects against multidrug-resistant organisms. These include MRSA, carbapenem-resistant *P. aeruginosa*, MDR *A. baumannii*, multi-resistant *N. gonorrhoeae*, and ciprofloxacin-resistant *S. aureus*. Notably, several AI-derived molecules exhibited equal or superior efficacy compared with standard-of-care antibiotics *in vitro*. Detailed antimicrobial descriptions of the top-performing compounds or peptides from each study are provided in **Supplementary Table 6**.

Together, the consistent ability of AI-designed candidates to achieve potent, broad-spectrum activity highlights the growing maturity of AI-driven discovery pipelines. These advances naturally lead into the next critical stage of antibiotic development, pre-clinical evaluation, where AI increasingly contributes to predicting synthesizability, toxicity, optimizing pharmacological profiles, and guiding the selection of compounds with the highest likelihood of translating into safe and effective therapeutics.[9,17,20]

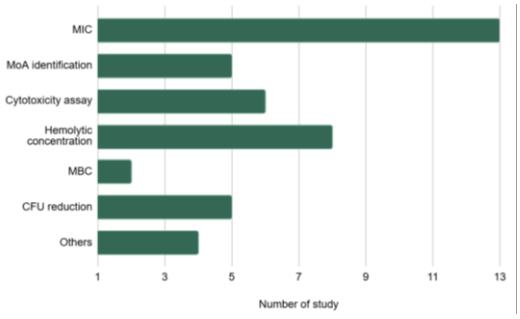

Figure 6. Distribution of *in vitro* assays

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AI-Assisted Predictive Toxicology and Pharmacokinetic Profiling

Across the included studies, AI-driven de novo AMP design has demonstrated strong capabilities in generating structurally novel compounds with high antibacterial potency and unexpectedly favorable toxicity profiles.[36,43,45,46] However most AI models were not explicitly designed to predict pharmacokinetic (PK) behavior or host toxicity, even though many AI-generated peptides subsequently exhibited low cytotoxicity and hemolysis during experimental testing.[5,8,41,43,45,46]

In almost all studies, the AI frameworks primarily optimized sequence features associated with antimicrobial activity, such as charge, hydrophobicity distribution, amphipathicity, or structural motifs. Toxicity-related parameters, such as hemolysis, mammalian cell cytotoxicity, serum stability, or metabolic degradation were rarely included as training objectives.[21,23] Only a minority used toxicity as an exclusion filter rather than a prediction target.[36]

The experimentally validated peptides consistently showed; low hemolytic activity, often with HC_{50} ≥ 512 µg/mL or <5% hemolysis at high concentrations[5,44,45]; minimal cytotoxicity toward common mammalian cell lines (HEK293, HepG2, HaCaT, HUVEC, etc.)[38,40,44], favorable therapeutic indices, often 20–200× above MIC values[44]; in vivo toxicity testing revealed no adverse effects, as indicated by normal organ weight ratios and maintained hematological profiles in mice[5]. These results indicate that while AI does not yet predict toxicity, it often generates peptides that fall within a physicochemical space associated with lower toxicity, likely due to optimization toward structural stability, reduced aggregation propensity, or avoidance of overly hydrophobic residues[44,45].

An additional aspect evaluated was the extent to which post-generation filtering incorporated pharmacokinetic considerations. In the studies analyzed, most post-generation filtering pipelines emphasized antimicrobial activity, structural feasibility, and novelty, while incorporating only limited and indirect pharmacokinetic (PK) considerations. Small-molecule studies occasionally used descriptors such as molecular weight, logP, or TPSA, but these were rarely embedded as optimization objectives[5,8]. Peptide-focused workflows relied mainly on physicochemical heuristics such as charge, hydrophobicity, helicity, or stability indices. These parameters influence solubility or protease susceptibility but do not constitute

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explicit ADME-PK evaluation.[21,23,36,40,41,43,44]

Khan (2025) was the only study implementing a clearly PK-informed filtering strategy. Early constraints on molecular weight and logP, followed by Lipinski, Veber, and Ghose rules, directly targeted permeability and oral bioavailability. Multi-stage selection using QSAR, docking, MD simulations, and a final MCDA framework integrated PK descriptors, ADMET predictions, and activity metrics to rank candidates with favorable bioavailability, low hERG liability, and metabolic stability. This represents the most comprehensive PK-integrated workflow among all reviewed studies.[5]

In contrast, other pipelines including Krishnan (2025), Wang (2025), Ortegon (2025), Kollen (2025), and multiple AMP-generation frameworks, applied filters centered on potency, safety, novelty, manufacturability, and peptide developability without incorporating serum stability, permeability, metabolic liability, or protein-binding assessment.[8,38,40,44] Even studies with extensive biological testing, such as Shen (2025), lacked PK-aware filtering or PK/PD modeling.[41]

These observations collectively demonstrate that pharmacokinetic evaluation in the reviewed literature was minimal. Therefore, current AI models cannot yet be considered capable of true PK prediction. At best, they provide optimized biophysical properties that may correlate weakly with PK performance, but this relationship remains largely untested.[5] Future development of multi-objective AI models integrating biological, toxicological, and PK data will be essential to advance de novo antibiotic design toward clinically translatable therapeutics.

Comparative *In Vivo* Efficacy of AI-Designed Antibiotics and Standard Therapies

AI-derived compounds demonstrated potent *in vivo* activity across diverse infection models, often matching or exceeding the effects of conventional antibiotics. A total of four studies conducted *in vivo* efficacy testing using murine models of infection, including skin abscess,[46] deep thigh infection,[46] sepsis,[5] vaginal infection,[8] and lung inflammation.[44] These models targeted a range of high-priority pathogens, notably *Acinetobacter baumannii*, *S. aureus* (including ciprofloxacin or methicilin-resistant strains), and *N. gonorrhoeae*.

As listed in **Supplementary Table 8**, *Acinetobacter baumannii* accounted for the largest share of *in vivo* validation efforts, featuring

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in four of eight murine infection models.[44-46] These studies demonstrated that AI-generated compounds, particularly AMPs, consistently achieved bacterial clearance levels comparable to or exceeding conventional treatments such as polymyxin B and levofloxacin.[46] In both skin abscess and deep thigh infection models, the AI-generated peptides reduced bacterial load by 3- to 4-fold and maintained this effect over multiple time points without inducing systemic toxicity, an encouraging outcome given the clinical toxicity associated with polymyxins.[46] Similarly, AMP-24, derived via a latent diffusion model, alleviated A. baumannii-induced lung pathology and inflammation, further supporting the utility of AI-derived peptides in managing Gram-negative infections with limited current options.[44]

In the case of *S. aureus*, including ciprofloxacin-resistant strains, AI-designed small molecules showed a more substantial therapeutic advantage.[5] SAK-2970, developed using a target-informed RNN model, achieved broad organ-level clearance in a murine sepsis model and improved survival rates significantly beyond those of ciprofloxacin.[5] Notably, even against resistant strains, the AI-derived compound preserved efficacy, with a 3-fold improvement in survival. Extended exposure studies with SAK-2970 revealed a significantly lower rate of resistance emergence over a 30-day period compared to ciprofloxacin, reinforcing growing concerns about the rapid resistance potential of fluoroquinolones.[5]

Comparable outcomes were observed for *N. gonorrhoeae* and MRSA infections. Although NG1, generated for vaginal *N. gonorrhoeae* infection, showed lower potency than ceftriaxone, it still produced a significant log-scale CFU reduction with no observable toxicity. Against MRSA, DN1 produced strong abscess clearance in a topical skin S aureus infection model.[8]

Importantly, no AI-designed agent tested *in vivo* demonstrated measurable toxicity or adverse physiological effects.[5,8,44,46] This includes assessments of weight loss, organ inflammation, and histological injury across models. Some, such as AMP-24, were explicitly shown to spare off-target tissues,[44] while others, like SAK-2970 and the peptides tested by Torres et al., maintained host stability even at therapeutic doses.[5,44] These findings emphasize the breadth of AI-designed antibiotic activity, extending across Gram-positive and Gram-negative bacteria, and across topical, systemic, and mucosal infection models.

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4. CONCLUSION

AI has emerged as a transformative tool in antibiotic discovery, addressing long-standing barriers of cost, time, and inefficiency in traditional pipelines. Across 13 studies, AI effectively supported target identification, generated diverse *de novo* antimicrobial candidates, and optimized lead molecules with strong *in vitro* and *in vivo* efficacy. While AI-generated antibiotics show promising preclinical potency and favorable toxicity signals, the current evidence base is limited by heterogeneous methods, sparse *in vivo*/PK data, incomplete reporting of AI model details, and potential publication bias. Continued refinement, particularly toward multi-objective optimization and PK-aware preclinical work, will be essential to draw firm conclusions about clinical transability.

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Supplementary Table 1. Thematic keywords used in search strategy for ai-based antibiotic discovery with preclinical evidence

Category	Keywords/Phrases
Artificial Intelligence	"artificial intelligence", "AI", "machine learning", "deep learning", "neural network", "generative model", "prediction model", "computational design"
Drug discovery	"de novo antibiotic", "de novo antimicrobial", "novel scaffold", " "de novo antimicrobial peptides"
Preclinical evidence	"preclinical", "*in vitro*", "wet lab", "*in vivo*", "mouse model", "murine infection"

Supplementary Table 2. Risk of Bias (RoB) for *in vitro* studies assessed with QUIN tool.

Criteria	Shen, 2025	Symzczak, 2023	Cao, 2025	Lin, 2025	Dean, 2021	Jin, 2025	Dean, 2020	Wang, 2025	Ortegon, 2025	Khan, 2025	Krishnan, 2025	Wang, 2025	Torres, 2024
1. Aim/objectives	2	2	2	2	2	2	2	2	2	2	2	2	2
2. Sample size	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
3. Sampling technique	1	2	2	2	1	2	1	2	1	2	2	2	1
4. Comparison group	2	2	2	2	0	2	2	2	2	2	2	2	2
5. Methodology	2	2	2	2	2	2	1	2	2	2	2	2	2
6. Operator details	0	1	0	1	0	0	0	0	1	1	1	1	1
7. Randomization	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
8. Outcome measurement	2	2	2	2	2	2	2	2	2	2	2	2	2
9. Outcome assessor	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
10. Blinding	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
11. Statistical analysis	2	2	2	2	2	2	2	2	2	2	2	2	2
12. Presentation of result	2	2	2	2	2	2	2	2	2	2	2	2	2
Total	13	15	14	15	11	14	12	14	14	15	15	15	14
Overall Bias	Low	Low	Low	Low	Mod	Low	Low	Low	Low	Low	Low	Low	Low

NA, not applicable.

Supplementary Table 3. Risk of Bias for *in vivo* studies assessed with SYRCLE's RoB tool

Criteria	Krishnan, 2025	Wang, 2025	Khan, 2025	Torres, 2024
1. Sequence generation	**Unclear**	**Unclear**	**Unclear**	**Unclear**
2. Similar baseline characteristics	Yes	Yes	Yes	Yes
3. Adequate allocation concealment	Yes	Yes	Yes	Yes
4. Random housing	NA	NA	NA	NA
5. Blinded caregiver	Yes	Yes	Yes	Yes
6. Random outcome assessment	**Unclear**	**Unclear**	**Unclear**	**Unclear**
7. Blinded outcome assessor	**Unclear**	**Unclear**	**Unclear**	**Unclear**
8. Detailed outcome data	Yes	Yes	Yes	Yes
9. Free of selective outcome reporting	Yes	Yes	Yes	Yes
10. Free of other sources of bias	Yes	Yes	Yes	Yes

*calculating a summary score for individual study using this tool were not recommended

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Supplementary Table 4. Summary of included study's characteristics

Study, Year	Type	Database	Number of data	AI Model	Generated	Lead	Wet Lab	*In vivo*
Jin, 2025	AMP	Uniref50	42,000,000	Seq-based	100	40	38	-
Szymzcak, 2025	AMP	Uniprot	225,244	cVAE	900	176	7	-
Kollen, 2025	Small molecules	ChEMBL, Natural Products	~365000	RNN-based	3436	48	11	-
Lin, 2023	AMP	APD3	>1000	WGAN-GP	8	8	8	-
Dean, 2021	AMP	Giant Repository of AMP Activity (GRAMPA)	6,760	Diffusion + Transformer VAE	100	38	2	-
Ortegon, 2025	Small molecules	ChemDiv & Enamine REAL	>1 billion	DGNN	194	156	8	-
Dean 2020	AMP	APD3 database	NA	VAE	NA	14	14	-
Cao, 2025	AMP	AMP + Non-AMP	38,540	Transformer (ProGen2) + RL	2,100,000	100	100	-
Shen, 2024	AMP	Metagenomic sequences	Billions	Deep Learning Classifier	2,109	85	39	-
Khrishnan, 2025	Small molecules	GDB-11, GDB-13, Enamine REAL	>45 million fragments	GNN + VAE	>36 million	24	7	2
Khan, 2025	Small molecules	ZINC (5.7M) + DHFR inhibitors	5.7 million compounds	RNN (LSTM), QSAR, DM	28,708	74	7	1
Wang, 2025	AMP	UniProt	>100000	Diffusion + Transformer VAE	41,107	40	40	2
Torres, 2024	AMP	APEX AMP	10 template	Transformer VAE + Bayesian optimization	Hundreds	100	86	10

Supplementary Table 5. Application of AI distribution across the antibiotic discovery pipeline

Study, year	Target identification	Lead Discovery and Optimization	AI-Assisted Preclinical Testing
Jin, 2025	No	Yes	Partially
Szymzcak, 2025	No	Yes	Partially
Kollen, 2025	No	Yes	Partially
Lin, 2023	No	Yes	Partially
Dean, 2021	No	Yes	No
Ortegon, 2025	Partially	Yes	Yes
Dean 2020	No	Yes	No
Cao, 2025	Yes	Yes	Partially
Shen, 2024	Yes	Yes	Partially

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Study, year	Target identification	Lead Discovery and Optimization	AI-Assisted Preclinical Testing
Khrishnan, 2025	Yes	Yes	Partially
Khan, 2025	No	Yes	Partially
Wang, 2025	No	Yes	Partially
Torres, 2024	No	Yes	No

Supplementary Table 7. Antimicrobial descriptions of top-performing compounds or peptides identified across studies

Study, Year	Types	AI-generated Compound	Bacterial Strain	MIC of Compound (µg/mL)	Control	Control MIC (µg/mL)
Jin, 2025	AMP	p21	E coli	2.2	Ampicilin / Polymyxin B	9.7 / 0.3
			P aeruginosa	2.2	Polymyxin B	0.3
			E faecalis	4.4	Ampicilin	4.4
		p30	S aureus	1.1	Ampicilin	0.5
			E faecalis	4.4	Ampicilin	4.4
Szymczak, 2023	AMP	Hydraganan-1	E coli	2.0	Pexiganan	4.0
			S aureus	8.0	Pexiganan	32.0
			A baumanii	4.0	Pexiganan	2.0
			P aeruginosa	32.0	Pexiganan	8.0
Kollen, 2025	Small molecule	D8	E coli	1.6	Nitrofurazone / Nitrofurantoin / Tetracycline	10.0 / 49.0 / 3.0
			S aureus	0.8	Nitrofurazone	18.8
			K pneumoniae	3.1		
			A baumanii	>200		
			P aeruginosa	>200		
			E cloacae	100.0		
Lin, 2023	AMP	GAN-pep 3	E coli	2	Polyphemusin I	0.7
			MSSA	6	Polyphemusin I	>50
			MRSA	45	Polyphemusin I	>50
			P aeruginosa	3	Polyphemusin I	>50
			P aeruginosa (carbapenem-resistant)	3		
Dean, 2021	AMP	p37	E coli	2		
			S aureus	8		
		p35	P aeruginosa	0,5		
Ortegon, 2025	Small molecule	Compound-5	E coli	0,5		
			P mirabilis	0,0625		
			K pneumoniae	4		
		Comopund-6	A baumanii	0.003		
Cao, 2025	AMP	Amphion 7	A baumanii	0.5 µmol/L		
			E cloacae	8.0 µmol/L		
			E coli	2.0 µmol/L		
			K pneumoniae	1.0 µmol/L		
			P aeruginosa	4.0 µmol/L		
			S enterica	4.0 µmol/L		
			B subtilis	0.5 µmol/L		

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Study, Year	Types	AI-generated Compound	Bacterial Strain	MIC of Compound (µg/mL)	Control	Control MIC (µg/mL)
			S aureus	8.0 µmol/L		
			E faecium	2.0 µmol/L		
Torres, 2024	AMP	Mammuthusin 2-3	A baumanii	4 µmol/L		
			E coli	4 µmol/L		
			K pneumoniae	8 µmol/L		
		Myolodonin 3-9	A baumanii	2 µmol/L		
			E coli	1 µmol/L		
			P aeruginosa	8 µmol/L		
Krishnan, 2025	Small molecule	NG1	N gonorrhoeae	0.5	Ceftriaxone, Vancomycin	
			N gonorrhoeae (multi resistant)	0.5		
			N meningitidis	0.5		
Khan, 2025	Small molecule	SAK-2970	S aureus	13.48	Ciprofloxacin	16
			S aureus (ciprofloxacin resistant)	13.48		
Wang, 2025	AMP	AMP-17	C neoformans	6.25	Ampicilin, Piperacilin, Amoxicilin, Ceftaroline fosamil, ceftriaxone	
			P aeruginosa	3.125		
			K pneumoniae	3.125		
			A baumanii	3.125		
			E coli	3.125		
		AMP 24	A baumanii	6.25		
			E coli	6.25		

Supplementary Table 8. Summary of *in vivo* studies, experimental models, and key outcomes.

Study, Year	Mouse Model	Target Bacteria	De Novo Antibiotic	Assay	Control	Result
Torres, et al. 2024	Skin abscess infection	A. baumannii	Mylodoniin-2-3, Equusin-4-6	Bacterial load (Day 2 & 4 post-treatment)	Polymyxin B, Levofloxacin	Bacterial load decreased 4-fold; no weight change, skin damage, or adverse effects.
	Deep thigh infection	A. baumannii	Mylodoniin-2-3, Mammuthusin-3-6	Bacterial load (Day 2 & 4 post-treatment)	Polymyxin B, Levofloxacin	Comparable to control with ~3-fold reduction; bacteriostatic effect by Day 4; no weight loss (non-toxic).

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Study, Year	Mouse Model	Target Bacteria	De Novo Antibiotic	Assay	Control	Result
Khan, et al. 2025	Murine sepsis	S. aureus	SAK-2970	CFU reduction (24h post-treatment)	Ciprofloxacin	Significant CFU reduction in blood, liver, spleen, kidney, lung; >70% survival vs. 50% with ciprofloxacin.
	Murine sepsis (drug-resistant)	Ciprofloxacin-resistant S. aureus	SAK-2970	Survival (96h) & bacterial load (24h)	Ciprofloxacin	~60% survival vs. 20% with ciprofloxacin; bacterial load reduced in liver, spleen, kidneys, blood, and lungs.
Krishnan et al., 2025	Vaginal infection	N. gonorrhoeae	NG1	CFU reduction (24h post-treatment)	Ceftriaxone	~3-log CFU reduction vs. vehicle; less potent than ceftriaxone; no observed toxicity.
	Skin abscess infection	MRSA	DN1	CFU reduction (24h post-treatment)	Fusidic acid	~10-fold CFU reduction vs. vehicle; no observed toxicity.
Wang, et al. 2025	Murine skin infection	A. baumannii	AMP-24	Histological analysis (H&E, Masson's) 24h post-treatment	None	Reduced inflammatory cell infiltration 4h post-treatment.
	Murine lung infection	A. baumannii	AMP-24	Histological analysis (H&E, Masson's) 24h post-treatment	None	Markedly alleviated lung inflammation and fibrosis; no immune infiltration in heart, liver, or kidney, suggesting low systemic toxicity.