



Review

AI-Driven Drug Repurposing for Multidrug-Resistant Infections: Computational Rigor, Translational Realities, and Strategic Future Directions

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Abstract

Antimicrobial resistance (AMR) is a rapidly escalating global health crisis, and multidrug-resistant (MDR) bacterial pathogens cause substantial morbidity, mortality, and economic burden. Conventional antibiotic-discovery pipelines remain slow, costly, and prone to high attrition, creating an urgent need for alternative strategies. Drug repurposing, which involves identifying new antimicrobial applications for approved or investigational agents, offers a pragmatic and cost-effective approach that leverages existing pharmacokinetic, safety, and formulation data. Advances in artificial intelligence (AI), including machine learning, deep learning, graph neural networks, and natural language processing (complemented by AI-adjacent computational approaches such as network pharmacology and molecular docking, which are treated here as distinct methods), have accelerated the high-throughput screening and prioritisation of repurposable compounds. This review critically evaluates AI-driven drug repurposing for MDR infections, addressing computational methodologies, data integration, experimental validation, translational bottlenecks, and regulatory considerations. The literature published between January 2020 and September 2026 was identified through searches of PubMed/MEDLINE, Scopus, and Web of Science, and each case-study drug is annotated for the role of AI and for the level of computational, in vitro, animal, and clinical evidence. We examine methodological limitations, including dataset bias, interpretability constraints, and pharmacokinetic gaps, and highlight the need for explainable AI and rigorous validation frameworks. Case studies of halicin, auranofin, phenothiazines, disulfiram, and clofazimine distinguish agents discovered by AI (halicin) from those whose antimicrobial activity was established by conventional screening or clinical use and illustrate both the promise and the translational hurdles of repurposed antimicrobials. We conclude by outlining strategic pathways for integrating AI predictions with experimental and clinical workflows, emphasising multidisciplinary collaboration to accelerate translation. By combining computational innovation with pharmacological and regulatory rigor, AI-driven repurposing constitutes a complementary strategy to de novo antibiotic discovery, with the potential to expand therapeutic options against MDR pathogens and strengthen global antimicrobial stewardship.

Keywords: antimicrobial resistance; drug repurposing; artificial intelligence; multidrug resistance; machine learning; translational pharmacology; WHO priority pathogens



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1. Introduction

Antimicrobial resistance (AMR) is among the most pressing public health challenges of the twenty-first century, threatening the effective treatment of infectious diseases worldwide [1,2]. Multidrug-resistant (MDR) pathogens, particularly the critical- and high-priority bacteria of the WHO Bacterial Priority Pathogens List (BPPL) 2024, such as carbapenem-resistant *Acinetobacter baumannii* and Enterobacterales, third-generation cephalosporin-resistant Enterobacterales, methicillin-resistant *Staphylococcus aureus*, and vancomycin-resistant *Enterococcus faecium* (groups that overlap substantially with the traditional ES-KAPE acronym [3,4]), pose major clinical challenges owing to their capacity to evade multiple antibiotic classes [5,6]. Current global estimates attribute 1.14 million deaths directly to bacterial AMR in 2021 (4.71 million associated deaths), and forecasts suggest 1.91 million attributable deaths annually by 2050 [2]; the earlier estimate for 2019 was 1.27 million attributable deaths [1]. The associated economic burden, including prolonged hospitalisation, increased intensive care unit (ICU) utilisation, and billions of dollars in healthcare expenditure, is compounded by productivity losses across affected populations [7,8]. Traditional antibiotic-development pipelines have proven insufficient to counter this evolving threat. Scientific complexity, high attrition rates, long development timelines, and limited commercial incentives have produced a sparse pipeline of novel antimicrobials [9,10]; the WHO analysis of the antibacterial pipeline concluded that progress toward innovative agents against priority pathogens remained insufficient [10]. Drug repurposing has therefore emerged as a pragmatic strategy for identifying new antimicrobial indications for approved or investigational drugs. By leveraging existing safety, pharmacokinetic, and formulation data, repurposing can reduce development costs, shorten timelines, and lower the risk of unexpected toxicity [11,12].

Historically, drug repurposing has relied largely on serendipitous clinical or in vitro observations. For example, the antirheumatic gold compound auranofin was subsequently found to exhibit activity against *Staphylococcus aureus* and vancomycin-resistant enterococci (VRE), prompting further targeted preclinical investigation [13]. Artificial intelligence (AI) has since transformed the field, enabling systematic, high-throughput screening of large chemical libraries and the integration of multi-dimensional biological data [14–16]. Machine learning (ML), deep learning (DL), graph neural networks (GNNs), network pharmacology, and natural language processing (NLP) collectively support predictive modelling of drug–pathogen interactions, uncovering antimicrobial activities that might otherwise remain undetected [17–20].

AI-driven approaches can further integrate chemical, genomic, transcriptomic, proteomic, and clinical datasets to produce prioritised candidates with greater translational likelihood [21,22]. Nonetheless, methodological limitations persist: dataset bias, overfitting, limited interpretability, and inadequate pharmacokinetic modelling remain significant hurdles [23–25]. Addressing these issues requires explainable AI, rigorous cross-validation, and integration with experimental pharmacology to ensure that computational predictions are biologically and clinically meaningful [25–27].

This review critically examines the current state of AI-driven drug repurposing for MDR bacterial infections, focusing on computational frameworks, data-integration strategies, translational bottlenecks, regulatory considerations, and ethical challenges. Drawing on developments from 2020–2026 identified through searches of PubMed/MEDLINE, Scopus, and Web of Science (the English-language literature published between January 2020 and September 2026, supplemented by manual screening of the reference lists of key papers), we outline practical pathways for accelerating the identification and clinical implementation of repurposed therapeutics, with the aim of strengthening global antimicrobial stewardship and addressing the unmet clinical needs posed by MDR pathogens.

Scope: What Is Meant by AI-Driven Drug Repurposing

In this review, AI-driven drug repurposing denotes the use of data-driven algorithms that learn statistical or representational patterns from data, namely supervised and unsupervised machine learning (ML), deep learning (DL) including graph neural networks (GNNs), and natural language processing (NLP) or knowledge-graph methods, to predict or prioritise new antimicrobial uses for existing approved or investigational compounds [14,17]. Structure-based methods (molecular docking and molecular dynamics, MD), classical quantitative structure–activity relationship (QSAR) modelling, and network-pharmacology analyses are computational but not intrinsically AI: they rely on physics-based scoring, hand-crafted descriptors, or curated interaction networks rather than learned representations [28,29]. Because these approaches are frequently combined with ML in integrated pipelines, they are discussed here as AI-adjacent methods, and each case study in Section 6 is annotated with the actual role played by AI in the discovery and evaluation of the candidate.

2. Global Burden of Multidrug-Resistant Infections

MDR bacterial infections constitute a growing global health and economic crisis. Global analyses estimate that bacterial AMR was directly responsible for 1.14 million deaths and associated with 4.71 million deaths in 2021 (compared with 1.27 million attributable deaths in 2019), with forecasts of 1.91 million attributable deaths annually by 2050 [1,2]. This burden falls disproportionately on low- and middle-income countries (LMICs), where limited access to effective antibiotics, inadequate infection control, and resource constraints magnify the impact of resistance [Table 1] [1,30].

Table 1. Major multidrug-resistant (MDR) pathogens and associated clinical burden and WHO Bacterial Priority Pathogens List 2024 category [5,6].

Pathogen	Common Infections	Key Resistance Mechanisms	Clinical Impact	WHO BPPL 2024 Priority Category (Resistance Phenotype)
<i>Staphylococcus aureus</i> (MRSA)	Skin infections, pneumonia, sepsis	<i>mecA</i> gene (β-lactam resistance)	High hospital-acquired infection rate	High (methicillin-resistant)
<i>Enterococcus faecium</i> (VRE)	Bloodstream infections, UTIs	<i>vanA/vanB</i> genes	Limited therapeutic options	High (vancomycin-resistant)
<i>Klebsiella pneumoniae</i>	Pneumonia, bloodstream infections	Carbapenemases (KPC, NDM)	High mortality in ICU	Critical (carbapenem-resistant and third-generation cephalosporin-resistant Enterobacterales)
<i>Acinetobacter baumannii</i>	Ventilator-associated pneumonia	Efflux pumps, OXA enzymes	Severe hospital outbreaks	Critical (carbapenem-resistant)
<i>Pseudomonas aeruginosa</i>	Burn infections, sepsis	Biofilm formation, efflux pumps	Frequent treatment failure	High (carbapenem-resistant)
<i>Enterobacter</i> spp.	UTIs, bacteraemia	AmpC β-lactamase production	Rising resistance globally	Critical (within third-generation cephalosporin-resistant and carbapenem-resistant Enterobacterales)
<i>Mycobacterium tuberculosis</i>	Pulmonary and extrapulmonary tuberculosis	<i>rpoB</i> mutations (rifampicin resistance)	Long, toxic treatment regimens	Critical (rifampicin-resistant)

The ESKAPE pathogens are of particular clinical concern given their propensity for rapid resistance acquisition and dissemination [3]. The WHO Bacterial Priority Pathogens List (BPPL) 2024 now provides the reference framework: carbapenem-resistant *A. baumannii*, third-generation cephalosporin-resistant and carbapenem-resistant Enterobacterales (including *K. pneumoniae* and *Enterobacter* spp.), and rifampicin-resistant *Mycobacterium tuberculosis* are classified as “critical”, whereas carbapenem-resistant *P. aeruginosa*, MRSA, and vancomycin-resistant *E. faecium* are classified as “high”-priority [5,6] (Table 1). The list updates and replaces the 2017 priority list [4], and the pathogens in this review are therefore discussed according to the BPPL categories rather than the ESKAPE acronym alone.

Beyond clinical outcomes, MDR infections impose substantial economic costs. Extended hospital stays, ICU admissions, and additional therapeutic interventions increase healthcare expenditure considerably; in the United States, the direct cost of treating hospitalised patients with MDR bacterial infections was estimated at approximately US\$4.6 billion in 2017 [8,31]. In the EU/EEA, an estimated 33,110 deaths in 2015 were attributable to infections with antibiotic-resistant bacteria [32]; low-resource settings face a compounded burden, as high mortality coincides with limited capacity for advanced care [1].

MDR infections also increase the risk of sepsis, organ failure, and prolonged mechanical ventilation, and antimicrobial resistance has been consistently associated with higher mortality, longer hospital stay, and higher costs [7]. Drivers of the rising prevalence of MDR pathogens include the overuse and misuse of antibiotics in human and veterinary medicine, inadequate infection control, and global travel facilitating pathogen dissemination [30], compounded by an antibacterial pipeline that remains insufficient in innovative agents against priority pathogens [9,10].

Collectively, these data illustrate that MDR infections represent both a clinical threat of high morbidity and mortality and an economic burden that strains healthcare systems worldwide, underscoring the need for accelerated discovery and deployment of effective antimicrobials, including AI-driven repurposing strategies capable of rapidly identifying viable candidates against high-priority pathogens.

3. Principles of Drug Repurposing in Antimicrobial Therapy

Drug repurposing, also termed drug repositioning, involves identifying new therapeutic indications for existing approved or investigational compounds. For MDR infections, repurposing offers a strategic advantage by leveraging existing pharmacological, safety, and formulation data to accelerate clinical translation [11,12]; unlike conventional antibiotic discovery, which requires extensive preclinical and clinical evaluation, repurposed agents may bypass some early-phase safety studies, reducing both development timelines and cost [11,33]. Mechanistically, repurposed drugs can act through several pathways, summarised below.

- **Direct antimicrobial activity.** Certain compounds exhibit intrinsic bactericidal or bacteriostatic effects against MDR pathogens; the antidiabetic candidate halicin, for example, inhibited a broad range of Gram-negative and Gram-positive bacteria, including carbapenem-resistant Enterobacteriaceae and *M. tuberculosis* in vitro and pan-resistant *Acinetobacter baumannii* in a mouse wound model, by dissipating the Δ pH component of the proton motive force [16].
- **Host-targeted modulation.** In principle, some repurposed drugs may enhance host defence or modulate inflammatory signalling to facilitate pathogen clearance; for bacterial infections this mechanism is less well established than direct or synergistic activity and is not the focus of the case studies below.
- **Synergistic enhancement of existing antibiotics.** Certain non-antibiotic compounds can potentiate antibiotic activity, for example by inhibiting efflux pumps and thereby

increasing intracellular antibiotic accumulation; phenothiazines, originally developed as antipsychotics, inhibit multidrug efflux-pump activity in *Staphylococcus aureus* [34]. Efflux inhibition cannot, however, explain potentiation of β -lactams in general, because resistance to this class is mediated mainly by β -lactamases and altered penicillin-binding proteins [30].

Several translational challenges remain. Achieving therapeutic concentrations at infection sites, particularly for intracellular and biofilm-associated pathogens, is a key limitation. Moreover, the pharmacokinetic (PK) and pharmacodynamic (PD) profiles of repurposed agents may be suboptimal for antimicrobial therapy, requiring dose optimisation or formulation adjustment [12,35]. Importantly, promising preclinical efficacy does not always translate into clinical success because of host–pathogen interactions, toxicity, and off-target effects.

AI-driven repurposing frameworks may help address some of these limitations by predicting compound efficacy, identifying synergistic combinations, and prioritising candidates based on integrated chemical, genomic, and phenotypic data [14–16]. Network-based analyses and multi-omics integration enable a systems-level understanding of drug–pathogen–host interactions, improving the likelihood of successful translation [19,21,29]. In sum, the principles of antimicrobial drug repurposing reflect a balance between computational prediction and empirical validation that, by systematically leveraging existing pharmacological knowledge while addressing PK/PD and mechanistic constraints, offers a viable, accelerated route to expanding the armamentarium against MDR pathogens [Table 2].

Table 2. Artificial intelligence and AI-adjacent computational approaches used in drug repurposing.

Method (AI or AI-Adjacent)	Key Characteristics	Application in Antimicrobial Discovery	Advantages	Limitations
Machine learning (AI)	Uses structured datasets	Predict antimicrobial activity	Interpretable models	Dataset bias, class imbalance, data leakage
Deep learning (AI)	Neural network architectures	Pattern recognition in chemical structures	High predictive accuracy	Low interpretability
Graph neural networks (AI)	Molecules represented as graphs	Predict drug–target interactions and whole-cell activity	Captures molecular topology	High computational cost; generalisation to novel scaffolds remains challenging
Network pharmacology (AI-adjacent)	Systems-level analysis	Identifies multi-target drug actions	Holistic biological view	Database dependency
Natural language processing (AI)	Text mining from the literature	Identifies hidden drug–pathogen relationships	Utilises a vast range of the literature	Data standardisation challenges
Molecular docking/MD (classical, non-AI comparator)	Physics-based scoring of ligand–target binding; needs a target structure	Prioritise approved drugs against defined bacterial targets	Mechanistic transparency; no training data required	Scoring-function errors; does not capture cell entry or efflux

4. Artificial Intelligence Methodologies

AI is increasingly used in drug repurposing to enable systematic, high-throughput prediction of potential antimicrobial agents against MDR pathogens [14,15,17]. It encompasses a suite of computational methods, including ML, DL, GNNs, network pharmacology (an AI-adjacent, network-based approach), and NLP, each offering distinct capabilities for identifying and prioritising repurposable drugs through the integration of chemical, genomic, proteomic, and clinical data, thereby extending analysis beyond the limits of traditional in vitro screening. Table 2 summarises the key characteristics, applications, advantages, and limitations of these approaches.

4.1. Machine Learning

Supervised ML algorithms, including random forests, support vector machines, and gradient boosting, are widely used to model structure–activity relationships and predict antimicrobial potential from chemical descriptors [17]. Trained on datasets of known active and inactive compounds, ML models can identify molecular features correlated with antimicrobial activity; their principal advantage is interpretability, as feature-importance analyses can highlight molecular motifs associated with efficacy. However, ML approaches remain sensitive to dataset bias and imbalance, which can produce overfitting or reduced generalisability [24,36], making robust cross-validation, data augmentation, and ensemble modelling essential to predictive reliability.

4.2. Deep Learning

Deep learning methods, a subset of machine learning, including convolutional neural networks (CNNs), recurrent neural networks (RNNs), and transformer architectures, can learn complex, high-dimensional representations of chemical structures and biological interactions with minimal reliance on manually engineered features [17,18]. CNN-based models can process molecular fingerprints or graph-derived embeddings to predict antimicrobial activity, while transformer-based models incorporate sequential biological or chemical information for more nuanced prediction. Despite strong predictive performance, DL models often lack transparency, complicating interpretability and clinical translation [27,37]; explainable AI (XAI) methods, such as attention visualisation and SHAP (Shapley Additive Explanations), have been proposed to address this limitation, and substructure-based explainable models have been used to identify a novel structural class of antibiotics [26].

4.3. Graph Neural Networks

Graph neural networks (GNNs) represent molecules as graphs, with nodes corresponding to atoms and edges to chemical bonds, enabling the modelling of topological and relational chemical properties critical to antimicrobial activity [16,18]. GNNs can learn task-specific molecular representations and have been used to prioritise structurally novel antibacterial chemotypes [16,26]; however, generalisation to unseen scaffolds remains challenging, and performance is typically lower under scaffold-based than random data splits [18,38]. GNNs require substantial computational resources and large, high-quality training datasets, limiting accessibility for some research groups.

4.4. Network Pharmacology

Network pharmacology provides a systems-level framework for evaluating drug–target–disease interactions [19,29]. By integrating protein–protein interaction networks, gene-expression data, and disease pathways, it identifies repurposable drugs that modulate multiple nodes within bacterial and host networks, facilitating identification of multi-target compounds or synergistic combinations potentially effective against complex MDR

infections. Network pharmacology is treated here as an AI-adjacent approach: graph-based analyses can be combined with ML, but network construction and scoring are not in themselves AI. Its principal limitations are dependence on the completeness and accuracy of network databases and the potential propagation of existing biases.

4.5. Natural Language Processing

Natural language processing (NLP) techniques enable extraction of latent knowledge from unstructured text, including scientific studies, clinical trial records, and patent databases [20,21]. Text-mining pipelines can identify overlooked drug–pathogen interactions, adverse-effect profiles, or off-target mechanisms relevant to repurposing; transformer-based language models such as BioBERT have improved the accuracy of biomedical entity recognition and relation extraction, accelerating candidate identification. Challenges remain in standardising terminology, handling incomplete data, and integrating textual insights with experimental datasets.

4.6. Integrated AI Pipelines

Effective AI-driven drug repurposing often combines machine learning, deep learning, and graph neural network predictions with insights from network pharmacology and natural language processing [14,15,17]. Integration of heterogeneous datasets, including chemical structures, transcriptomic, proteomic, and metabolomic profiles, and the biomedical literature, can enhance predictive performance and reduce false positive predictions. However, important translational bottlenecks remain, as many AI-predicted candidates fail during in vivo evaluation because of pharmacokinetic limitations, complex host–pathogen interactions, or toxicity [12,27,35]. Federated learning and transfer learning strategies have emerged to improve generalisability across diverse datasets while preserving patient and institutional privacy [39].

4.7. Limitations and Critical Appraisal

Dataset bias, model overfitting, and insufficient external validation continue to limit reliability, while black-box DL and GNN models complicate mechanistic interpretation and undermine regulatory confidence [27,37]. These issues are addressed by the validation practices in Section 4.8, together with explainable AI, multi-omics validation, PK/PD integration (Section 7.1), and interdisciplinary collaboration among computational scientists, pharmacologists, microbiologists, and clinicians. When coupled with experimental validation and pharmacological realism, AI methodologies nonetheless offer powerful tools for prioritising repurposable drugs, complementing rather than replacing traditional antibiotic-development pipelines.

4.8. Model Validation and Computational Rigor

Because computational rigor is central to the credibility of AI-driven repurposing, five validation issues merit explicit attention. (i) Data splitting and scaffold splitting. Random train–test splits place close structural analogues in both sets and therefore overestimate performance on new chemistry; scaffold-based (Bemis–Murcko) or time-based splits hold out entire chemical series and give a more realistic estimate of generalisation to novel chemotypes, usually with lower scores [18,38]. (ii) Data leakage. Leakage occurs when information from the test set influences training, for example through duplicate or near-duplicate structures, salt or tautomer variants of the same compound, or feature selection and hyperparameter tuning performed on the full dataset; it is a recognised driver of irreproducible machine-learning results and should be prevented by structure standardisation, deduplication, and strict separation of training, validation, and test data [24,25]. (iii) Class imbalance. Antibacterial screens yield few actives among many inactives, so

accuracy and ROC-AUC can be misleadingly high; precision–recall curves, enrichment metrics, and calibrated probabilities, combined with resampling or cost-sensitive learning, are more informative [36]. (iv) External and prospective validation. Retrospective cross-validation should be complemented by testing on independent external datasets and, ultimately, by prospective experimental testing of held-out predictions, reporting the number of compounds tested and the confirmed hit rate, as in the studies discussed in Section 6 [16,27]. (v) Empirical in vitro validation. In silico predictions, however rigorously validated computationally, remain hypotheses until they are confirmed experimentally, and in vitro testing is the final and most significant validation of the computational work. Prioritised compounds should be tested against reference strains and relevant MDR clinical isolates by standardised susceptibility testing (minimum inhibitory and minimum bactericidal concentrations, time–kill kinetics, and checkerboard assays for synergy), together with cytotoxicity assays on mammalian cells to establish selectivity and, where feasible, mechanism-of-action studies, using appropriate controls and replicates, before progression to animal models [16,27]. Reporting negative results and releasing code, data splits, and model versions would further improve reproducibility.

5. Data Sources and Integration

The success of AI-driven drug repurposing depends heavily on the quality, diversity, and integration of available data. Large-scale chemical, biological, and clinical datasets underpin ML, DL, and network-based models, enabling accurate prediction of antimicrobial potential against MDR pathogens [14,17].

5.1. Chemical Databases

Comprehensive resources such as DrugBank, ChEMBL, PubChem, ZINC, and the Protein Data Bank (PDB) provide molecular structure, bioactivity, and target information [40,41], supplying standardised descriptors and physicochemical properties for model training. High-quality annotation of active versus inactive compounds is essential to minimise dataset bias, particularly for predicting novel antimicrobial activity; incomplete or inconsistent annotation remains a persistent challenge that can compromise generalisability [24].

5.2. Biological and Genomic Databases

Resources such as the Gene Expression Omnibus (GEO), Sequence Read Archive (SRA), UniProt, and BioGRID provide transcriptomic, proteomic, and protein–protein interaction data relevant to bacterial pathogens and host immune responses [21]. Multi-omics integration allows AI models to account for complex pathogen–host interactions, revealing candidates that act via direct antimicrobial activity or host-targeted modulation, although discrepancies in experimental protocols, platform variability, and incomplete annotation introduce noise requiring careful preprocessing.

5.3. Clinical and Real-World Data

Electronic health records, clinical trial repositories, adverse-event databases, and pharmacovigilance systems contribute real-world evidence to drug repurposing, although EHR-derived data are prone to bias [23]. Linking clinical efficacy, dosage regimens, and safety profiles to AI predictions enhances translational relevance, while federated-learning approaches enable integration of multi-institutional datasets while preserving patient privacy [39].

5.4. Multi-Omics Integration Challenges

Integrating heterogeneous chemical, genomic, proteomic, metabolomic, and clinical datasets is essential to fully realise AI's potential, providing a systems-level view of drug–pathogen–host interactions and enabling prediction of synergistic mechanisms and off-target effects [21,22]. Persistent challenges include data sparsity, missing values, heterogeneous measurement units, and variable quality; matrix factorisation, graph-based integration, and embedding strategies have been employed to harmonise datasets and reduce bias [21,29].

5.5. Data Curation and Validation

Rigorous data curation and preprocessing, including standardisation of chemical structures, normalisation of gene expression data, and validation against experimentally verified datasets, are essential for generating reliable AI predictions [25]. Benchmarking on external validation sets and prospective experimental testing remain critical to reproducibility and translational feasibility. Overall, effective AI-driven repurposing depends on comprehensive, curated, and integrated datasets spanning chemical, biological, and clinical domains, with data quality and heterogeneity remaining key determinants of model accuracy and translational success.

6. Case Studies of Repurposed Antimicrobials: The Role of AI and the Level of Evidence

Not all repurposed antimicrobials discussed in the literature were identified by AI. Halicin is a clear example of AI-based antibacterial discovery, whereas the antibacterial or synergistic properties of auranofin, phenothiazines, disulfiram, sertraline, and clofazimine were established through experimental investigation or clinical experience, with computational methods playing at most a supporting or retrospective role. Each case study below therefore states the role of AI explicitly (Table 3) and the level of computational, in vitro, animal, and clinical evidence (Table 4).

Table 3. Case-study repurposed drugs with antimicrobial activity and the documented role of AI in their identification.

Drug	Original Indication	Target Pathogen(s) and Evidence	Mechanism of Antimicrobial Action	Documented Role of AI
Halicin	c-Jun N-terminal kinase inhibitor investigated for diabetes	Enterobacteriaceae and <i>M. tuberculosis</i> (in vitro); pan-resistant <i>A. baumannii</i> and <i>C. difficile</i> (mouse models) [16]	Dissipates the Δ pH component of the proton motive force [16]	AI-discovered: deep learning model applied to the Drug Repurposing Hub [16]
Auranofin	Rheumatoid arthritis	Gram-positive bacteria including MRSA (in vitro) [13]	Disrupts thiol-redox homeostasis, including thioredoxin reductase [13]	None verified; activity established experimentally
Phenothiazines	Antipsychotic drugs	<i>S. aureus</i> (efflux inhibition, in vitro) [34]	Inhibition of multidrug efflux-pump activity; not a general explanation for β -lactam potentiation	None verified; properties known before AI methods
Disulfiram	Alcohol dependence	<i>Mycobacterium tuberculosis</i> (in vitro) [42]	Copper-dependent bactericidal activity [42]	Not established
Sertraline	Antidepressant	<i>S. aureus</i> , <i>E. coli</i> , <i>P. aeruginosa</i> (in vitro) [43]	Incompletely characterised	Not established
Clofazimine	Leprosy (antimycobacterial)	Mycobacteria including MDR tuberculosis [44]	Redox cycling, reactive oxygen species, disruption of respiration [44]	None: clinical repurposing within mycobacterial disease

Table 4. Evidence hierarchy for the case-study candidates (“Yes” = evidence cited in this review; “–” = no evidence identified among the sources cited in this review, which does not imply absence in the wider literature).

Drug	Computational (AI) Evidence	In Vitro	Animal	Clinical
Halicin	Yes	Yes	Yes (mouse)	–
Auranofin	–	Yes	–	–
Phenothiazines	–	Yes	–	–
Disulfiram	–	Yes	–	–
Sertraline	–	Yes	–	–
Clofazimine	–	Yes	–	Yes (leprosy; MDR-TB regimens)

6.1. Halicin (AI-Discovered)

Halicin, initially investigated as a c-Jun N-terminal kinase inhibitor for diabetes, was identified by a deep learning model (a directed message-passing neural network) trained on growth-inhibition data and applied to the Drug Repurposing Hub [16,41]. It inhibited the growth of a broad range of bacteria in vitro, including *M. tuberculosis* and carbapenem-resistant Enterobacteriaceae, and was effective in mouse models of pan-resistant *A. baumannii* wound infection and *Clostridioides difficile* infection [16]. Mechanistically, it is reported to dissipate the Δ pH component of the proton motive force, a mechanism distinct from that of most conventional antibiotics [16]. The evidence is preclinical: clinical efficacy data have not, to our knowledge, been reported, and the pharmacokinetic, toxicity, and formulation work required for systemic use remains to be completed, underscoring the importance of integrating AI predictions with experimental pharmacology.

6.2. Auranofin (Antibacterial Activity Established Experimentally)

Auranofin, a gold-containing compound approved for rheumatoid arthritis, has bactericidal activity against Gram-positive bacteria including MRSA and is proposed to act by disrupting thiol-redox homeostasis, including inhibition of thioredoxin reductase [13]. Its antibacterial activity was established experimentally; a study in which AI-based screening prioritised auranofin as an antibacterial could not be identified, and AI should not be credited with its discovery. Translation requires pharmacokinetic and safety evaluation at exposures relevant to antibacterial therapy, because the approved dosing regimen was designed for a different indication.

6.3. Phenothiazines (Known Efflux-Inhibitory Activity; No Verified AI Role)

Phenothiazines such as chlorpromazine and thioridazine, originally developed as antipsychotics, inhibit multidrug efflux-pump activity in *S. aureus* [34]. Their antibacterial and resistance-modifying properties were described well before the current generation of AI methods, and no evidence was found that they were identified by AI-driven network pharmacology. The efflux-inhibition evidence concerns efflux substrates such as fluoroquinolones; it should not be extrapolated to β -lactams, for which resistance is mediated predominantly by β -lactamases and altered penicillin-binding proteins [30], so any potentiation of β -lactams by phenothiazines requires separate mechanistic evidence and cannot be ascribed to efflux inhibition alone. Central nervous system adverse effects and the concentrations required limit systemic use, emphasising the need for dose optimisation and targeted delivery strategies.

6.4. Disulfiram (AI Role Not Established)

Disulfiram, a long-established alcohol-aversion therapy, kills *M. tuberculosis* in a copper-dependent, synergistic manner in vitro [42]. This activity was characterised experimentally, and an AI-based prediction of disulfiram as an antibacterial could not be verified. Achieving effective tissue concentrations while limiting copper-related toxicity remains a translational challenge, illustrating the gap between in vitro activity and clinical applicability.

6.5. Sertraline and Clofazimine (No AI Discovery Role)

Sertraline, an antidepressant, inhibited *S. aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* in vitro and enhanced the activity of several antibiotics [43]; the inhibitory concentrations reported (tens of µg/mL for reference strains) likely exceed those achievable at antidepressant doses. Clofazimine is an antimycobacterial (anti-leprosy) drug already used in regimens for multidrug-resistant tuberculosis, with activity linked to redox cycling, generation of reactive oxygen species, and disruption of respiration [44]; its use in MDR tuberculosis reflects clinical repurposing within the same therapeutic area rather than AI-based discovery. Both illustrate the diversity of repurposing routes and require careful PK/PD optimisation and toxicity evaluation before wider deployment.

6.6. AI Versus Classical Computational Approaches: Hit Rates and Limitations

Comparison with classical methods requires care. Prospective AI screens report variable hit rates: in the halicin study, 8 of 23 model-selected ZINC15 compounds that were tested inhibited bacterial growth [16], whereas in the *A. baumannii* study roughly 9 of 240 selected compounds were active (about 4%) [45]. Docking-based repurposing studies usually test smaller sets: a 2026 study that docked approved drugs against essential bacterial proteins tested 14 top-ranked drugs, of which lifitegrast showed the most notable activity, seen in combination with a membrane-permeabilising agent [46]. Per-study hit rates from small, pre-selected sets are not directly comparable with those from screens of thousands to millions of compounds, because they depend on library size and pre-filtering, the activity threshold, target-based versus whole-cell readouts, and publication bias towards positive outcomes; to our knowledge, no prospective head-to-head benchmark of AI versus docking and molecular dynamics for antibacterial repurposing has been reported. Classical methods retain clear strengths (mechanistic transparency, no need for large training sets, explicit modelling of a defined target and binding mode), but they are prone to false positives from scoring-function inaccuracies [28] and do not themselves account for bacterial cell entry and efflux. AI models trained on whole-cell phenotypes capture such properties implicitly and can screen far larger chemical spaces, but they depend on training-data coverage, offer limited mechanistic insight, generalise less reliably to novel scaffolds (Section 4.8), and have rarely been compared with classical methods in matched prospective studies. Moreover, most AI-identified antibacterial hits, including halicin, have not progressed to clinical evaluation. The most defensible position is therefore complementary use: AI for prioritisation at scale, structure-based methods for mechanistic hypotheses, and experimental validation against predefined success criteria. Recent work, including a 2025 generative model that designed novel antibacterial compounds de novo [47] and a 2024 foundation model for clinician-centred drug repurposing [22], extends these approaches, but prospective clinical evidence is still lacking.

6.7. Critical Analysis

Collectively, these case studies show that AI can reveal antibacterial activity in previously overlooked compounds (halicin), but that most repurposed antimicrobials discussed

here came to attention through experimental screening or clinical experience, so the contribution of AI should be judged compound by compound (Table 3). The evidence base remains predominantly preclinical (Table 4), and the translational hurdles (tissue exposure, host–pathogen interactions, off-target effects) are treated in Section 7 [12,35]. AI therefore complements rather than substitutes for experimental pharmacology.

7. Translational and Regulatory Considerations

Clinical translation of AI-identified repurposed drugs for MDR infections requires careful consideration of pharmacokinetics, pharmacodynamics, safety, and regulatory compliance. Computational predictions alone cannot guarantee clinical efficacy; translational strategies must bridge the gap between in silico identification and patient-level outcomes [12,27,35].

7.1. Pharmacokinetic and Pharmacodynamic Optimisation

Pharmacokinetic and pharmacodynamic (PK/PD) considerations are critical in antimicrobial drug repurposing. Key PK/PD indices include the free drug AUC-to-MIC ratio (fAUC/MIC), free drug peak concentration-to-MIC ratio (fC_{max}/MIC), and the percentage of the dosing interval above the MIC (fT > MIC). Successful repurposing requires achieving the PK/PD exposure threshold associated with the desired antimicrobial effect at the infection site while maintaining safe drug concentrations. The relevant threshold depends on the antimicrobial agent and its mechanism of action. This is particularly important for intracellular, biofilm-associated, or tissue-specific infections, where repurposed candidates such as halicin and auranofin may require dose optimisation, formulation, or targeted delivery to achieve effective concentrations in vivo [35]. Population PK modelling and physiologically based pharmacokinetic (PBPK) simulation can guide dosing strategies and inform trial design, particularly for drugs with narrow therapeutic windows [35].

7.2. Preclinical Validation

Experimental validation remains indispensable. In vitro assays, including minimum inhibitory concentration (MIC) testing, time-kill studies, and biofilm disruption assays, provide direct evidence of antimicrobial activity [35], while in vivo models, such as murine sepsis or infection models, evaluate efficacy, toxicity, and tissue distribution. AI predictions can prioritise candidates for these studies, improving efficiency and reducing resource use, although discrepancies between model organisms and human physiology necessitate cautious interpretation and iterative refinement of preclinical protocols [12].

7.3. Clinical Trial Design

Regulatory agencies, including the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA), require robust evidence of safety and efficacy even for repurposed drugs. Trials for repurposed antimicrobials often adopt adaptive or phase II-focused designs to expedite evaluation while safeguarding patient safety; combination approaches, such as phenothiazines with conventional antibiotics, require additional trials to assess synergy and potential adverse interactions. Incorporating AI predictions into trial design can optimise candidate selection, dosing regimens, and patient stratification.

7.4. Regulatory Considerations

Although repurposed drugs may benefit from existing safety data, approval for a new indication still mandates adherence to Good Clinical Practice (GCP), demonstrable efficacy, and pharmacovigilance planning. Intellectual-property considerations can influence commercial viability: off-patent drugs may face limited financial incentive, whereas proprietary compounds may offer the dual benefit of repurposing and extended market pro-

tection. Public–private partnerships can help offset financial risk and support translation in resource-limited settings.

7.5. Critical Analysis

While AI accelerates candidate identification, translational success ultimately depends on integrating computational insight with rigorous pharmacological, clinical, and regulatory evaluation. Off-target toxicity, PK constraints, and regulatory barriers confirm that AI functions as a complementary tool rather than a standalone solution [27,37], reinforcing the need for interdisciplinary collaboration among computational scientists, microbiologists, pharmacologists, and regulatory experts to translate promising AI predictions into safe, effective therapies for MDR infections.

7.6. Strength of Evidence for the Case-Study Candidates

Table 4 maps each case-study candidate to the available computational, in vitro, animal, and clinical evidence among the sources cited in this review. Only halicin was discovered by AI, and it is supported by computational, in vitro, and animal evidence but not by clinical evidence. Auranofin, phenothiazines, disulfiram, and sertraline are supported here by in vitro evidence only, and clofazimine is the only candidate with established clinical use in mycobacterial disease. Consequently, none of the candidates is supported by clinical evidence as an AI-identified antibacterial repurposed drug, and claims of translational readiness should be made accordingly.

8. Limitations and Ethical Challenges

AI-driven drug repurposing offers substantial opportunities to combat MDR infections, but several limitations and ethical challenges must be addressed to ensure translational relevance, reproducibility, and equitable access.

8.1. Methodological Limitations

Dataset bias, interpretability, overfitting, and the limited integration of PK/PD parameters are discussed in Sections 4.7, 4.8 and 7.1 and are not repeated here. Two further limitations are specific to repurposing. First, prospective studies mainly report the few successful hits, so published success may overstate the true hit rate of AI pipelines (Section 6.6). Second, many approved non-antibiotics inhibit bacteria only at concentrations above those achievable with their approved dosing, which limits clinical relevance regardless of predictive accuracy [12].

8.2. Translational Constraints

Translational constraints (tissue penetration, biofilm-associated resistance, off-target toxicity, and regulatory evidence requirements) are detailed in Sections 6 and 7. The practical implication is that computational predictions should be treated as hypotheses to be tested against predefined experimental and PK/PD success criteria before clinical development.

8.3. Ethical and Equity Considerations

Ethical challenges include transparency, accountability, and global equity. The black-box nature of certain AI models can obscure decision-making, raising concerns about clinical accountability and patient safety [37]. Because training datasets are often disproportionately derived from high-income countries, AI models may have limited generalisability to low- and middle-income countries, where the burden of antimicrobial resistance is substantial [1,23]. This geographic imbalance underscores the need for representative datasets and rigorous validation across diverse populations and healthcare settings. Equity considerations should also extend beyond model development to the accessibility, afford-

ability, and implementation of repurposed therapies. Particular attention is warranted for off-patent medicines and agents with limited commercial incentives, as their potential clinical value may not translate into availability in resource-constrained settings without appropriate investment, regulatory support, and sustainable supply mechanisms. Ensuring that AI-driven antimicrobial drug repurposing addresses the needs of populations disproportionately affected by antimicrobial resistance is therefore essential to maximising its global public health benefit and avoiding further disparities in access to effective therapies.

8.4. Addressing the Challenges

Mitigating these limitations requires a multi-pronged strategy: implementing explainable AI to improve interpretability, expanding and diversifying datasets to reduce bias, integrating PK/PD modelling to enhance translational fidelity, and conducting prospective experimental validation to confirm computational predictions [27,37,39]. Collaborative international frameworks and open access platforms can further facilitate equitable dissemination of AI-driven repurposing findings, ensuring that high-burden regions benefit from these innovations.

AI-driven repurposing is transformative but not a panacea; its success depends on careful methodological design, rigorous validation, ethical deployment, and cross-disciplinary collaboration, factors that, if recognised early in the development pipeline, enhance the likelihood of producing safe, effective, and globally accessible therapies against MDR infections.

9. Future Directions

AI-driven drug repurposing for MDR infections has demonstrated significant promise, yet several avenues remain to enhance efficacy, translational success, and global impact, with future strategies expected to integrate computational innovation with mechanistic pharmacology, experimental validation, and clinical implementation.

9.1. Precision Antimicrobial Therapy

Integrating AI with patient-specific data—pathogen genomics, host immunogenomics, and microbiome composition—can enable precision antimicrobial therapy, optimising therapeutic selection, reducing off-target toxicity, and minimising secondary resistance [21,22]. Multi-omics profiling combined with AI could further identify patient subpopulations most likely to benefit from specific repurposed agents, facilitating targeted trials and improved outcomes.

9.2. Combination Therapy and Synergy Prediction

AI is increasingly applied to predict synergistic drug combinations; by modelling network pharmacology and multi-target interactions, it can identify combinations that potentiate existing antibiotics, overcome resistance mechanisms, or reduce effective doses [12,29]. Future research should systematically evaluate AI-predicted synergies in vitro and in vivo, alongside PK/PD modelling to ensure clinical feasibility.

9.3. Explainable and Interpretable AI

Enhancing regulatory confidence and clinical adoption will require future AI pipelines to prioritise explainable AI. Techniques such as attention visualisation, Shapley values, and graph interpretability can clarify the mechanistic basis of predictions, enabling clinicians and regulators to understand how and why specific candidates are prioritised [26,27,37]—a transparency requirement that is particularly critical in high-stakes areas such as MDR infection, where clinical decisions directly affect patient outcomes.

9.4. Integration with Experimental and Clinical Workflows

Bridging computational prediction with empirical validation remains a key challenge. Future efforts should develop integrated AI–experimental pipelines in which predictions are iteratively tested, refined, and validated in laboratory and animal models before advancing to clinical trials [12,35], with incorporation of real-world clinical data into AI models further refining predictive accuracy and accelerating translational timelines [21,39].

9.5. Global Accessibility and Equity

Addressing MDR infections requires globally accessible solutions. Future initiatives should prioritise open access AI platforms, international data sharing, and collaborative frameworks to ensure that AI-driven repurposing benefits regions with the highest resistance burden, including LMICs [1,2,37], supported by policies that incentivise repurposing of off-patent drugs and public–private partnerships.

9.6. Emerging Opportunities

Next-generation strategies include integrating AI with synthetic biology, antimicrobial peptide design, and biofilm-targeting therapies; AI may also predict repurposable adjuvants that enhance host immunity or modulate microbial virulence factors, expanding therapeutic options beyond conventional antimicrobials [14,47]. Combined, these innovations position AI-driven repurposing to reshape the antimicrobial landscape by offering scalable, rapid, and effective interventions against MDR pathogens—an approach whose future depends on precision, interpretability, integration, and equity, realised through synergistic computational–experimental workflows and strategic global collaboration.

10. Conclusions

The growing prevalence of MDR infections represents a critical global health threat with profound clinical and economic consequences [1,2,8]. Traditional antibiotic-discovery pipelines have proven insufficient to address this challenge, necessitating innovative approaches such as drug repurposing, which leverages existing pharmacological knowledge, safety data, and formulations to identify new antimicrobial indications with accelerated timelines and reduced development costs relative to de novo discovery [11,12].

AI has emerged as a transformative tool in this effort, enabling high-throughput prediction of drug–pathogen interactions, integration of multi-dimensional chemical and biological data, and prioritisation of candidates with translational potential [14,15,17]. ML, DL, GNNs, network pharmacology, and NLP each provide complementary strengths in uncovering repurposable agents and predicting synergistic combinations [14,15,17], and when integrated with experimental validation, PK/PD modelling, and clinical trial design, AI enhances the likelihood of successful translation to effective MDR therapies [12,27,35].

Case studies of halicin, auranofin, phenothiazines, disulfiram, sertraline, and clofazimine illustrate both the promise and the limits of AI-driven repurposing: only halicin was discovered by AI [16], whereas the antibacterial activity of auranofin, phenothiazines, disulfiram, sertraline, and clofazimine was established experimentally or clinically [13,34,42–44]. Translational and regulatory challenges (data quality, model interpretability, validation rigor, pharmacokinetic constraints, and ethical considerations) must be addressed to ensure clinical feasibility, safety, and equitable access (Tables 3 and 4) [27,37,39].

Looking forward, the future of AI-driven drug repurposing lies in precision antimicrobial therapy, explainable AI, combination-therapy prediction, and integrated computational–experimental workflows [14,21,26,27], supported by global collaboration and open access data platforms, particularly in regions with high MDR burden [1,2]. By addressing current methodological, translational, and ethical challenges, AI-driven repurposing offers a

scalable, accelerated approach to expanding the antimicrobial armamentarium, complementing existing stewardship strategies, and ultimately mitigating the growing threat of MDR infections. Its success will depend on integrating computational innovation, rigorous experimental validation, and globally equitable implementation to realise meaningful clinical outcomes against MDR pathogens.

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