Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/24553
DOI URL: http://dx.doi.org/10.21474/IJAR01/24553

RESEARCH ARTICLE

ARTIFICIAL INTELLIGENCE INMODERN MICROBIOLOGY: ADVANCES IN DIAGNOSTICS, RESISTANCE PREDICTION AND ANTIMICROBIAL DISCOVERY

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Manuscript Info

Manuscript History
Received: 08 September 2026
Final Accepted: 10 October 2026
Published: November 2026

Key words:-
artificial intelligence; machine learning; deep learning; antimicrobial resistance; antibiotic discovery; clinical microbiology

Abstract

Antimicrobial resistance (AMR) was responsible for an estimated 1.14 million deaths worldwide in 2021, and conventional growthbased susceptibility testing still requires 18 to 24 hours of incubationto obtain results. In this context, Artificial intelligence (AI) is increasingly being integrated into microbiological research, diagnostics and clinical practi ce. This review discusses how machine learning and deep learning are applied in microbial diagnosis, AMR prediction, antimicrobial discover y, microbiome research and genome design, and examines the evidence for their effectiveness. Image classification models have identified microorganisms in cropped images of Gram-stained blood cultures with 94.9% accuracy. Models based on MALDI-TOF mass spectra have shown moderate accuracy in predicting resistance, with AUROC values of 0.74 to 0.80 across three common bacterial species. Deep learning has identified antibiotic candidates that reduced bacterial loads in mouse models, and generative AI has designed novel compounds against Neisseria gonorrhoeae and methicillin-resistant Staphylococcus aureus (MRSA). DNA-trained language models have generated bacteriophage designs, although only 16 of 285 synthesized candidates produced viable phages. Most of the evidence, however, comes from retrospective datasets, singlecentre studies and animal experiments. Genomic resistance models perform better on strains closely related to their training data than on genetically diverse ones.Only 5% of machine learning decision-support tools for infectious diseases have been evaluated with real world clinical data. Data bias, limited interpretabilit y and unclear regulation restrict adoption. At present, AI is most useful as a supportive tool for screening and prioritizing candidates. Routine clinical use requires prospective, multicenter trials.

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Introduction:-

Microbiology laboratories generate large volumes of data that require careful analysis and interpretation. Routine diagnostic work involves examining stained smears, culture plates, mass spectrometry profiles and wholegenome sequences. Research groups sequence tens of thousands of metagenomes and screen chemical libraries that hold millions of compounds. Machine learning fits these tasks because it finds statistical patterns in large labeled datasets, and microbiology has accumulated many of them.

The global burden of bacterial antimicrobial resistance remains substantial. In 2021, an estimated 4.71 million deaths were associated with bacterial resistance, of which 1.14 million were directly attributable to it. These figures are projected to rise to 8.22 million associated deaths and 1.91 million attributable deaths annually by 2050 (Naghavi et al., 2024).AI research is increasingly focused on improving diagnostic speed, guiding appropriate treatment selection and accelerating the discovery of new antimicrobial drugs, all of which are essential for reducing the burden of antimicrobial resistance.

Several recent reviews have discussed the growing role of AI in microbiology (Wong et al., 2023; Cesaro et al., 2025; Xu et al., 2025). However, Xu et al. (2025) noted that many of these applications are still at an exploratory stage and require further validation. In the present review, we examine the major applications of AI in microbiology and assess the strength of evidence supporting each one, distinguishing between retrospective data analysis, laboratory experiments, animal studies and clinical investigations. The discussion primarily covers peer-reviewed publications from 2023 to 2026, along with earlier studies that established the foundations of these approaches. This article follows a narrative review approach, with studies selected based on their relevance and contribution to the subject rather than through a systematic literature search.

Diagnostics and image analysis:-

Microscopy is one area where deep learning has reached useful accuracy in a routine microbiology task. Smith et al. (2018) developed a convolutional neural network using 25,488 images of Gram-stained positive blood cultures, which were further divided into 100,213 image segments. The model classified bacterial morphologies with an overall accuracy of 94.9%, achieving an area under the curve (AUC) above 0.98 for all categories. When tested on 189 complete slides without human intervention, it demonstrated high sensitivity and specificity in identifying Gram-positive cocci in clusters (93.2% and 97.2%, respectively) and Gram-negative rods (96.3% and 98.1%, respectively). However, although sensitivity for Gram-positive cocci in chains and pairs reached 98.4%, specificity was lower at 75.0%, indicating a tendency to over-identify this morphology.Staining and imaging conditions vary between laboratories, so each laboratory would need to confirm the model on its own slides.

Smith and Kirby (2020) examined the application of image-based AI across several areas of microbiology, including stained smears, parasite preparations, blood films, histological sections and agar plates. They proposed that AI could be used for preliminary image screening and classification, while microbiologists would verify the findings and make the final diagnosis. They also pointed out that, once developed, image-analysis software can be operated at relatively low additional cost. This approach could reduce the routine workload in diagnostic laboratories by allowing AI to classify straightforward images and identify uncertain cases for further examination by microbiologists (Smith and Kirby, 2020).

Imaging-based approaches may also help reduce the time required for antimicrobial susceptibility testing. Zagajewski et al. (2023) developed a deep learning model to analyse single-cell microscopy images of Escherichia coli exposed to antibiotics. The method produced results after just 30 minutes of antibiotic exposure, compared with the 18–24 hours generally required for conventional growth-based testing. The classification accuracy was approximately 80% in the abstract and exceeded 84% in independent holdout experiments. The model was initially trained using the laboratory strain MG1655 and subsequently evaluated on six bloodstream isolates with varying levels of ciprofloxacin resistance. Ciprofloxacin exposure produced distinct morphological differences between susceptible cells and untreated controls, whereas co-amoxiclav induced less pronounced changes. Although the findings demonstrate the potential of imaging and deep learning for rapid susceptibility testing, the study remains a proof of concept because it examined only one bacterial species and four antibiotics.

Resistance prediction:-

Antimicrobial susceptibility results play an important role in selecting appropriate treatment. Therefore, early prediction of resistance can help guide timely therapeutic decisions. Researchers have explored different AI-based

approaches using three major types of data: MALDI-TOF mass spectra, whole-genome sequences and gene expression profiles.

MALDI-TOF mass spectrometry is already used in many clinical laboratories for bacterial identification, making the resulting protein spectra a readily available source of data for AI-based resistance prediction. Weis et al. (2022) developed the DRIAMS database, which contains more than 300,000 mass spectra and over 750,000 antimicrobial resistance phenotypes collected from four institutions. Machine learning models trained on these data predicted resistance with AUROC values of 0.80 for Staphylococcus aureus, 0.74 for Escherichia coli and 0.74 for Klebsiella pneumoniae. In a retrospective analysis involving 63 patients with invasive infections, the predictions could have influenced treatment decisions in nine cases, eight of which would have resulted in more appropriate treatment. Although these findings indicate the potential clinical value of MALDI-TOF-based resistance prediction, the small patient sample limits the strength of the evidence (Weis et al., 2022). Wong et al. (2023) similarly reported that the discriminatory performance of these classifiers generally remains around an AUROC of 0.7 and suggested that values above 0.9 may be necessary for reliable clinical application.

Whole-genome sequences provide a broader range of genetic information for predicting antimicrobial resistance. Ren et al. (2022) developed four machine learning models, namely logistic regression, support vector machine, random forest and convolutional neural network, using 987 Escherichia coli genomes obtained from Giessen. The models were subsequently evaluated using an independent collection of 1,509 publicly available genomes. Cross-validation AUC values ranged from approximately 0.69 to 0.96 across four antibiotics, with random forest models generally showing the best performance. However, the public test dataset was highly imbalanced, with unequal representation of resistant and susceptible isolates, making it difficult to assess how reliably the models would perform on genetically diverse bacterial populations (Ren et al., 2022).

Hu et al. (2024) compared four machine learning approaches with a conventional machine learning baseline and the rule-based tool ResFinder across 78 species–antibiotic datasets. Their findings showed that machine learning models performed better when the test strains were genetically similar to those used during training, whereas ResFinder was more effective in predicting resistance among genetically divergent strains. Prediction performance was more consistent for macrolides and sulfonamides but varied considerably for beta-lactams and tetracyclines. The authors emphasised the need for further model optimisation before clinical application, particularly when analysing strains that differ genetically from the training dataset (Hu et al., 2024). This limitation is particularly relevant during outbreaks, when newly emerging strains may have genetic characteristics that are poorly represented in existing training datasets.

Gene expression data provide another source of information for predicting antimicrobial resistance. Khaledi et al. (2020) analysed the genomic and transcriptomic profiles of 414 drug-resistant clinical isolates of Pseudomonas aeruginosa. Their machine learning models achieved sensitivity and predictive values of 0.8–0.9 or higher for four antimicrobials. Incorporating gene expression data further improved prediction performance for all tested drugs except ciprofloxacin. However, transcriptomic analysis is more expensive than genome sequencing, and its additional diagnostic value needs to be weighed against the cost and practical requirements of routine clinical testing (Khaledi et al., 2020).

Antimicrobial discovery:-

AI-based approaches have shown particularly promising results in antimicrobial discovery, with several studies reporting encouraging laboratory findings. However, translating computational predictions into effective and safe antimicrobial drugs remains a major challenge.Stokes et al. (2020) developed a neural network using bacterial growth inhibition data and used it to screen more than 107 million molecules from the ZINC15 database. Of the 23 predicted compounds selected for laboratory testing, eight showed antibacterial activity and had structures distinct from those of previously known antibiotics. One of these compounds, halicin, exhibited activity against Mycobacterium tuberculosis and carbapenem-resistant Enterobacteriaceae. It also showed efficacy against Clostridioides difficile and pan-resistant Acinetobacter baumannii infections in mouse models. In a separate study, Liu et al. (2023) screened approximately 7,500 compounds for their ability to inhibit A. baumannii and used the resulting data to train a machine learning model. This approach led to the identification of abaucin, a narrow-spectrum antibiotic that interferes with lipoprotein trafficking through the LolE protein. Abaucin demonstrated efficacy in a mouse model of wound infection. These studies illustrate how AI-based screening can identify structurally distinct antimicrobial candidates, although further investigation is required to establish their safety and effectiveness in humans.

Wong et al. (2024) investigated the use of explainable AI in antimicrobial discovery by initially screening 39,312 compounds for antibacterial activity and toxicity to human cells. They then employed ensembles of graph neural networks to predict these properties across a library of 12,076,365 compounds. Of the 3,646 predicted candidates, 283 were selected for experimental testing. The investigation identified a new structural class of compounds with selective activity against methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci. These compounds showed limited development of bacterial resistance and reduced bacterial loads in mouse models of skin and thigh infections. The study demonstrates how AI-based screening can accelerate the identification of antimicrobial candidates while considering both antibacterial activity and potential toxicity to human cells (Wong et al., 2024).

Generative AI approaches can go beyond screening existing chemical libraries by designing entirely new molecules with potential antibacterial activity. Krishnan et al. (2025) employed two generative strategies to produce more than 36 million novel compounds with predicted antibacterial properties. In the fragment-based approach, laboratory testing identified antibacterial activity in 7 of 66 compounds against Neisseria gonorrhoeae and 19 of 130 compounds against Staphylococcus aureus. The lead compound NG1 showed a minimum inhibitory concentration (MIC) of 0.5 μg/mL against N. gonorrhoeae ATCC 49226 and reduced bacterial load by approximately three log units in a mouse model of vaginal infection. Another candidate, DN1, exhibited an MIC of 4 μg/mL against both methicillin-susceptible and methicillin-resistant S. aureus and reduced bacterial load by approximately tenfold in a mouse skin infection model, with an effect comparable to fusidic acid. However, experimental validation was limited by the challenges of chemical synthesis. Of the 24 custom-synthesised compounds, only seven demonstrated selective antibacterial activity (Krishnan et al., 2025).

Santos-Júnior et al. (2024) explored metagenomic data for the discovery of antimicrobial peptides by analysing 63,410 metagenomes and 87,920 prokaryotic genomes. This work led to the development of AMPSphere, a database containing 863,498 non-redundant antimicrobial peptides. Of the 100 peptides selected for chemical synthesis and experimental evaluation, 79 showed antibacterial activity in vitro, including 63 that were effective against pathogenic bacteria. Further investigations indicated that these peptides exerted their antibacterial effects primarily by disrupting bacterial cell membranes (Santos-Júnior et al., 2024).

Torres et al. (2025) used the APEX 1.1 model to screen 233 archaeal proteomes for potential antimicrobial peptides. The model evaluated 193,331,608 candidate peptides and predicted 12,623 to have antimicrobial activity, which the authors named archaeasins. Of the 80 archaeasins synthesised and tested, 75 (over 93%) showed activity against at least one pathogen. Archaeasin-73 reduced the Acinetobacter baumannii load in a mouse skin abscess model to a level comparable to polymyxin B at early time points. However, this effect declined by day 4, and the peptides failed to prevent infection progression in a neutropenic thigh model. In addition, seven peptides showed both haemolytic activity and cytotoxicity against HEK293T cells at concentrations of 64 μmol/L or below (Torres et al., 2025). These findings indicate that high in vitro activity does not necessarily translate into efficacy in animal models. Wan et al. (2024) identified poor selectivity, unfavourable physicochemical properties, unclear mechanisms of action and high synthesis costs as major challenges in the development of antimicrobial peptides.

Microbiome analysis and genome-scale models:-

Microbiome datasets are highly dimensional, often containing many more features than samples, which increases the risk of overfitting. Przymus et al. (2025) reviewed the use of neural network models in microbiome research and reported that deep learning can identify functional genes, microbial interactions and host–microbiome dynamics. However, they emphasised that biological variability requires approaches tailored to specific applications to achieve reliable and generalisable results. This limitation is also evident in antimicrobial resistance prediction, where models often perform well on training datasets but show reduced accuracy when applied to independent datasets.

Genome language models also offer opportunities for designing biological sequences. Nguyen et al. (2024) developed Evo, a model with 7 billion parameters and a context length of 131,072 tokens, trained on prokaryotic and bacteriophage genomes. Without task-specific training, Evo predicted the effects of mutations on bacterial proteins with performance comparable to that of protein language models. Fine-tuned versions of the model were used to generate CRISPR-Cas9 systems, one of which demonstrated DNA-cleaving activity in vitro comparable to SpCas9. However, at the whole-genome level, the generated sequences lacked several conserved marker genes, indicating limitations in their ability to reproduce essential genomic features (Nguyen et al., 2024).

Building on this approach, King et al. (2026) fine-tuned Evo 1 and Evo 2 using approximately 15,000 Microviridae sequences, with the bacteriophage ΦX174 serving as the template. Of the 302 curated candidate genomes, 285 were synthesised and assembled, resulting in 16 viable phages. These included 12 of 227 candidates generated by Evo 1 (5.3%) and 4 of 58 generated by Evo 2 (6.9%). The designed phages inhibited the growth of Escherichia coli C and E. coli W. Furthermore, a cocktail of these phages was able to overcome resistance in two ΦX174-resistant E. coli C strains that were not inhibited by a cocktail of natural ΦX174-like phages (King et al., 2026). Although the success rate was low and the host range limited, the findings demonstrate that genome language models can generate functional phage genomes, supporting their potential application in developing phage-based treatments against antimicrobial-resistant bacteria.

Summary of the evidence:-

Table 1 summarises the key findings of these studies. However, none of them has reported patient outcomes from a prospective clinical trial.

Table 1. Representative studies and the level of evidence they provide

Application	Study	Main result	Evidence level
Gram stain classification	Smith et al., 2018	94.9% accuracy on image crops; 189 whole slides read	Retrospective image dataset
Rapid susceptibility from single-cell images	Zagajewski et al., 2023	Result after 30 minutes; about 80% single-cell accuracy	Laboratory study, one species
Resistance from MALDI-TOF spectra	Weis et al., 2022	AUROC 0.74 to 0.80 for three species	Retrospective, four institutions
Resistance from genomes	Hu et al., 2024	78 species-antibiotic datasets; accuracy depends on strain relatedness	Benchmark of published methods
Small-molecule antibiotic discovery	Stokes et al., 2020; Wong et al., 2024	New compounds active in mouse infection models	Preclinical
Generative antibiotic design	Krishnan et al., 2025	NG1 cut gonococcal load about 3 logs; DN1 cut MRSA load about 10-fold	Preclinical
Peptide mining	Santos-Júnior et al., 2024; Torres et al., 2025	79 of 100 and 75 of 80 peptides active in vitro	In vitro and mouse models
Genome language models	King et al., 2026	16 viable phages from 285 synthesized designs	Laboratory proof of concept

Challenges and limitations:-

Data availability and evaluation. Training datasets are often drawn from a limited number of settings. A review of 60 machine learning-based clinical decision-support systems for infectious diseases found that 88% were developed using data from high-income countries. Furthermore, 95% were evaluated using performance measures such as sensitivity and specificity, while only three systems (5%) were tested using data from clinical practice (Peiffer-Smadja et al., 2020). As this review covered studies published up to July 2019, these figures may not reflect more recent developments. Cesaro et al. (2025) further noted that high-quality labelled datasets are often held in proprietary databases or are expensive to generate. They also emphasised that models trained on data from limited patient populations or specific diagnostic assays may not perform reliably when applied to other settings.

Generalization. Hu et al. (2024) demonstrated this limitation in their benchmark study. The performance of machine learning-based resistance predictors declined when they were tested on genetically divergent strains, and the best-performing method varied according to the bacterial species and antibiotic examined. These findings indicate that laboratories should validate adopted models using their own clinical isolates before applying them in routine practice.

Interpretability. The difficulty in interpreting how AI models arrive at their predictions raises concerns about their reliability and acceptance. Although post hoc explanation methods can provide insights into model outputs, they have limitations (Cesaro et al., 2025). Wong et al. (2024) addressed this issue by grouping predicted antimicrobial candidates according to shared structural features, providing a basis for explaining their selection.

Laboratory validation. AI-generated predictions should be treated as hypotheses that require experimental validation. Although archaeasins showed an in vitro hit rate of over 93%, they failed to control infection in a neutropenic thigh model (Torres et al., 2025). Similarly, the development of compounds identified by generative models in the gonorrhoea and MRSA study was limited by the number of candidates that could be synthesised for testing (Krishnan et al., 2025). Generative models may also produce outputs that appear biologically plausible but are incorrect. Therefore, experimental validation remains essential before any predicted candidate can be considered for further development (Cesaro et al., 2025).

Accuracy thresholds. MALDI-TOF-based resistance classifiers generally achieve AUROC values of around 0.7. Wong et al. (2023) suggested that performance above 0.9 may be required for reliable clinical application. Until this level of accuracy is achieved, AI-based diagnostic tools are likely to serve primarily as supportive tools in clinical decision-making (Wong et al., 2023).

Regulation. By the time of their 2025 policy article, Warraich et al. (2025) reported that the United States Food and Drug Administration (FDA) had authorised nearly 1,000 AI-enabled medical devices. This figure covered medical applications across all fields and was not specific to microbiology. The authors emphasised the need for regular, site-specific monitoring of device performance after approval, along with dedicated evaluation of large language models. Accordingly, laboratories adopting AI-based classifiers should establish ongoing monitoring procedures to assess their performance as local bacterial strains, instruments and reagents change.

Conclusions:-

The strongest evidence for AI applications in microbiology comes from image analysis and antimicrobial resistance prediction, where several retrospective studies have reported consistent findings. Evidence for antimicrobial discovery and genome design remains more limited, with results largely based on laboratory experiments and animal models. Current findings support the use of AI tools for preliminary slide screening, prioritizing candidate antimicrobial compounds and predicting potential resistance. However, all predictions require review and confirmation by laboratory personnel. At present, the evidence is insufficient to support replacing conventional culture-based antimicrobial susceptibility testing with AI-based methods.

Four steps would strengthen the field:-

Prospective multicentre clinical trials should evaluate patient outcomes rather than relying solely on diagnostic performance measures such as sensitivity and specificity.

Shared training and test datasets should be developed using representative data from low- and middle-income countries, which remain underrepresented in current datasets.

External validation on strains that differ from the training set, with results reported by species and antibiotic.

Full reporting of failed experiments, such as peptides that were active in vitro but ineffective in animals.

With these in place, AI methods have a realistic path to routine use in diagnostic laboratories and drug discovery programs.

Acknowledgements:-

The author sincerely acknowledges the contributions of researchers and scientific communities whose published work in artificial intelligence, microbiology, computational biology and antimicrobial research provided the scientific foundation for this review.

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