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ARTIFICIAL INTELLIGENCE–DRIVEN PREDICTION OF ANTIMICROBIAL RESISTANCE USING INTEGRATED CLINICAL, WHOLE-GENOME SEQUENCING, AND METAGENOMIC DATA: A PROSPECTIVE MULTICENTER COHORT STUDY

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ABSTRACT

Background: Antimicrobial resistance (AMR) is a major global health challenge that compromises effective treatment of infectious diseases and increases morbidity, mortality, and healthcare costs. Artificial intelligence (AI) has emerged as a promising approach for rapid prediction of resistance phenotypes using large-scale biological and clinical datasets. The objective of the study is to develop and validate a multimodal AI framework integrating clinical, whole-genome sequencing, and metagenomic data for accurate prediction of antimicrobial resistance in a prospective multicenter cohort.

Materials and Methods: This prospective multicenter study enrolled 2,492 patients with confirmed bacterial infections from five tertiary-care hospitals. Clinical characteristics, antimicrobial susceptibility testing results, whole-genome sequencing data, and metagenomic profiles were collected. Multiple machine-learning models, including Logistic Regression, Random Forest, XGBoost, and Deep Neural Networks (DNNs), were trained and evaluated. Model performance was assessed using area under the receiver operating characteristic curve (AUC), accuracy, sensitivity, specificity, and F1-score. External validation was performed in an independent cohort of 742 patients.

Result: Among 2,492 patients, 1,014 (40.7%) exhibited antimicrobial-resistant infections. Prior antibiotic exposure (adjusted OR=3.12, 95% CI: 2.61–3.74), ICU admission (adjusted OR=2.46, 95% CI: 1.98–3.06), blaNDM positivity (adjusted OR=5.28, 95% CI: 4.02–6.93), and reduced microbiome diversity (adjusted OR=2.39, 95% CI: 1.91–2.99) were independent predictors of resistance. The multimodal DNN achieved the highest performance with an AUC of 0.957 (95% CI: 0.947–0.967), accuracy of 91.8%, sensitivity of 92.6%, specificity of 90.9%, and F1-score of 0.917. External validation confirmed excellent predictive performance (AUC=0.943; accuracy=90.4%).

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Conclusion: Integration of clinical, genomic, and metagenomic data through a multimodal AI framework enables highly accurate prediction of antimicrobial resistance. Such models may facilitate earlier therapeutic decision-making and strengthen precision antimicrobial stewardship strategies.

Keywords: Antimicrobial resistance, Artificial intelligence, Machine learning, Whole-genome sequencing, Metagenomics, Deep learning, Predictive modelling, Precision medicine, Antimicrobial stewardship, Multi-omics.

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the most pressing global public health threats of the twenty-first century. The increasing prevalence of multidrug-resistant bacterial pathogens compromises the effectiveness of existing antimicrobial therapies, leading to prolonged hospital stays, increased healthcare expenditures, treatment failures, and higher mortality rates.[1,2] The complexity of AMR evolution is driven by a combination of ecological, evolutionary, and clinical factors, including antibiotic overuse, horizontal gene transfer, microbial adaptation, and the dissemination of resistance determinants across healthcare and community settings.[3,4] Consequently, rapid and accurate prediction of resistance phenotypes has become a critical priority for improving antimicrobial stewardship and patient outcomes.

Conventional antimicrobial susceptibility testing (AST) remains the clinical gold standard for determining bacterial resistance profiles. However, culture-based methods often require 24–72 hours or longer to generate results, delaying targeted therapy and potentially contributing to inappropriate empirical antibiotic use.[5] Advances in whole-genome sequencing (WGS) have enabled the identification of resistance-associated genes and mutations, providing opportunities for earlier prediction of antimicrobial susceptibility. Nevertheless, genomic approaches alone may not fully capture the dynamic interactions between microbial communities, host factors, and environmental influences that contribute to resistance emergence.[6,7]

Recent developments in artificial intelligence (AI) and machine learning (ML) have transformed the analysis of complex biomedical datasets. These computational approaches can identify non-linear relationships within large-scale clinical and molecular datasets, enabling highly accurate prediction of disease outcomes and microbial characteristics.[8,9] Several studies have demonstrated the utility of ML algorithms for predicting AMR directly from genomic data, achieving promising diagnostic performance across multiple bacterial species and antimicrobial classes.[10,11] Systematic reviews and meta-analyses further suggest that AI-based AMR prediction models may substantially improve the speed and accuracy of resistance detection compared with conventional approaches.[12]

Simultaneously, metagenomic sequencing has emerged as a powerful tool for characterizing entire microbial communities and their resistomes without requiring culture. Metagenomic analyses can detect uncultivable organisms, resistance genes, and microbial interactions that may influence treatment response.[13] Integration of metagenomic information with genomic and clinical datasets offers a more comprehensive representation of resistance mechanisms than any single data source alone. Recent machine-learning frameworks have demonstrated the feasibility of extracting clinically relevant AMR signatures from metagenomic data, highlighting the potential of multi-omics approaches for precision infectious disease management.[9,10]

Despite these advances, most published AI-based AMR prediction studies have relied on retrospective datasets, single-center cohorts, or isolated genomic features, limiting their generalizability and clinical applicability.[14] Prospective multicenter investigations integrating clinical variables,

WGS-derived genomic markers, and metagenomic profiles remain scarce. Furthermore, the real-world performance of multimodal AI systems across diverse patient populations and healthcare settings has not been adequately evaluated.

Therefore, the present prospective multicenter cohort study aims to develop and validate an integrated artificial intelligence framework for predicting antimicrobial resistance using clinical characteristics, whole-genome sequencing data, and metagenomic profiles. By leveraging multimodal data sources, this study seeks to enhance predictive accuracy, facilitate earlier therapeutic decision-making, and support precision antimicrobial stewardship strategies in contemporary clinical practice.

MATERIALS AND METHODS

Study Design and Participants: This prospective multicenter cohort study was conducted between January 2026 and December 2027 across five tertiary-care hospitals. Consecutive patients aged ≥18 years with clinically suspected bacterial infections requiring microbiological investigation were enrolled after obtaining informed consent. Patients with incomplete clinical records, contaminated specimens, or inadequate sequencing data were excluded. The study protocol was approved by the institutional ethics committees of all participating centers.

Clinical, Genomic, and Metagenomic Data Collection: Demographic characteristics, comorbidities, prior antimicrobial exposure, laboratory parameters, infection source, and clinical outcomes were extracted from electronic health records using a standardized data collection form. Clinical specimens underwent routine culture and antimicrobial susceptibility testing according to current Clinical and Laboratory Standards Institute (CLSI) guidelines. Whole-genome sequencing was performed on culture-positive isolates using the Illumina platform to identify resistance genes, virulence factors, and genomic variants. Parallel shotgun metagenomic sequencing was conducted directly on clinical specimens to characterize microbial community composition and resistome profiles.

Artificial Intelligence Model Development: Clinical variables, genomic features, and metagenomic signatures were integrated into a multimodal dataset. After preprocessing and normalization, the dataset was randomly divided into training (70%), validation (15%), and testing (15%) cohorts. Multiple machine-learning algorithms, including Random Forest, Extreme Gradient Boosting (XGBoost), and Deep Neural Networks, were developed to predict antimicrobial resistance phenotypes. Feature importance was assessed using SHapley Additive exPlanations (SHAP) to enhance model interpretability.

Outcome Measures and Statistical Analysis: The primary outcome was accurate prediction of phenotypic antimicrobial resistance. Model performance was evaluated using area under the receiver operating characteristic curve (AUC), accuracy, sensitivity, specificity, precision, and F1-score. Continuous variables were summarized as mean ± standard deviation or median (interquartile range), while categorical variables were expressed as frequencies and percentages. Statistical analyses were performed using R version 4.4 and Python version 3.12. A two-sided p-value <0.05 was considered statistically significant.

RESULTS

A total of 2,684 patients were screened across five tertiary-care centers during the study period. Following exclusion of 192 patients due to incomplete clinical data (n=76), contaminated cultures (n=49), or insufficient sequencing quality (n=67), 2,492 patients were included in the final analysis. Antimicrobial resistance (AMR) was identified in 1,014 (40.7%) patients, whereas 1,478 (59.3%) exhibited susceptible phenotypes.

[Table 1] summarizes the baseline characteristics of patients with resistant and susceptible infections. Patients with antimicrobial-resistant infections were significantly older and had higher rates of ICU admission, prior antibiotic exposure, comorbidities, prolonged hospitalization,

and 30-day mortality compared with those with susceptible infections. [Table 2] presents the multivariable logistic regression analysis identifying independent predictors of antimicrobial resistance. Prior antibiotic exposure, ICU admission, resistance-associated genes (blaNDM and blaCTX-M), reduced microbiome diversity, and higher comorbidity burden emerged as significant determinants of resistant infections.

[Table 3] compares the predictive performance of the evaluated machine-learning algorithms. The multimodal deep neural network achieved the highest discrimination and overall classification performance, significantly outperforming conventional statistical and machine-learning approaches. [Table 4] demonstrates the external validation and subgroup performance of the developed prediction model. Consistently high predictive accuracy across microbiological and clinical subgroups indicates strong generalizability and robustness of the proposed framework. [Table 5] ranks the most influential variables contributing to antimicrobial resistance prediction according to SHAP analysis. Genomic resistance markers, previous antimicrobial exposure, and microbiome diversity measures were identified as the dominant predictive features.

[Figure 1] illustrates the predictive performance achieved using different combinations of clinical, genomic, and metagenomic data. Progressive improvement in model discrimination was observed with multimodal data integration, with the highest performance achieved when all three data sources were combined. [Figure 2] presents the decision-curve analysis comparing the clinical utility of the multimodal deep-learning model with conventional logistic regression. Across a wide range of threshold probabilities, the proposed model demonstrated greater net clinical benefit, supporting its potential value in real-world antimicrobial stewardship. [Figure 3] shows the calibration performance of the final prediction model. The close agreement between predicted and observed resistance probabilities across risk deciles indicates excellent calibration and reliability of the model's risk estimates.

Table 1: Baseline Characteristics of the Study Population

Variable	Resistant (n=1014)	Susceptible (n=1478)	p-value
Age, years (mean ± SD)	61.4 ± 15.2	54.1 ± 16.3	<0.001
Male sex, n (%)	612 (60.4)	782 (52.9)	<0.001
ICU admission, n (%)	348 (34.3)	272 (18.4)	<0.001
Prior antibiotic exposure, n (%)	596 (58.8)	382 (25.8)	<0.001
Charlson Comorbidity Index ≥4, n (%)	418 (41.2)	378 (25.6)	<0.001
Length of stay, days (median, IQR)	14 (9–22)	8 (5–13)	<0.001
30-day mortality, n (%)	153 (15.1)	87 (5.9)	<0.001

Table 2: Multivariable Logistic Regression Analysis for Predictors of Antimicrobial Resistance

Variable	Adjusted OR	95% CI	p-value
Prior antibiotic exposure	3.12	2.61–3.74	<0.001
ICU admission	2.46	1.98–3.06	<0.001
blaNDM positivity	5.28	4.02–6.93	<0.001
blaCTX-M positivity	3.54	2.83–4.43	<0.001
Reduced microbiome diversity	2.39	1.91–2.99	<0.001
Charlson Index ≥4	1.78	1.41–2.25	<0.001
Age (>65 years)	1.42	1.15–1.76	0.001

Table 3: Performance of Machine-Learning Models in Internal Validation Cohort

Model	AUC (95% CI)	Accuracy (%)	Sensitivity (%)	Specificity (%)	F1 Score
Logistic Regression	0.821 (0.801–0.841)	78.9	75.3	81.2	0.774
Random Forest	0.904 (0.891–0.917)	85.6	84.7	86.1	0.853
XGBoost	0.932 (0.921–0.943)	88.4	87.2	89.1	0.881

Deep Neural Network	0.957 (0.947–0.967)	91.8	92.6	90.9	0.917

Table 4: External Validation and Subgroup Analysis

Cohort	AUC (95% CI)	Accuracy (%)
Overall validation cohort	0.943 (0.929–0.957)	90.4
Gram-negative infections	0.951 (0.938–0.964)	91.2
Gram-positive infections	0.926 (0.903–0.949)	88.7
ICU patients	0.938 (0.918–0.958)	89.4
Non-ICU patients	0.947 (0.931–0.963)	91.1
Bloodstream infections	0.954 (0.938–0.970)	92.1

Table 5: Top Predictive Features Ranked by SHAP Importance

Rank	Feature	Mean SHAP Value
1	blaNDM gene	0.271
2	Prior antibiotic exposure	0.248
3	blaCTX-M gene	0.229
4	Shannon diversity index	0.201
5	ICU admission	0.185
6	mecA gene	0.167
7	Age	0.143
8	Charlson Comorbidity Index	0.132

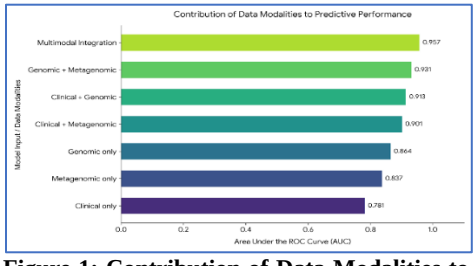

Figure 1: Contribution of Data Modalities to Predictive Performance

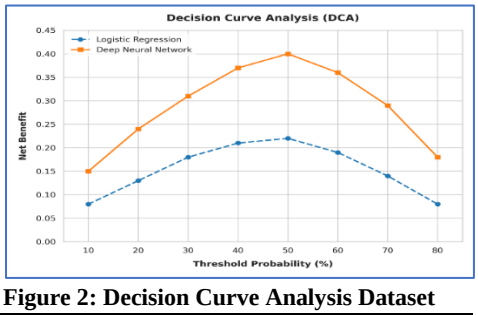

Figure 2: Decision Curve Analysis Dataset

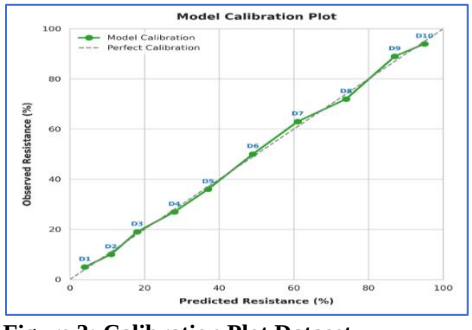

Figure 3: Calibration Plot Dataset

DISCUSSION

The present prospective multicenter study developed and externally validated a multimodal artificial intelligence (AI) framework integrating clinical variables, whole-genome sequencing data, and metagenomic profiles for the prediction of antimicrobial resistance (AMR). The proposed deep neural network demonstrated excellent predictive performance, achieving an AUC of 0.957 in internal validation and 0.943 in external validation, with consistently high sensitivity, specificity, and calibration across diverse patient subgroups. These findings highlight the value of integrating host, microbial, and resistome-level information into a unified predictive model and support the growing role of AI-driven precision diagnostics in antimicrobial stewardship.

Our findings are consistent with the growing body of evidence demonstrating the utility of machine learning for AMR prediction. Kim et al,[1] emphasized that AI models can substantially improve resistance prediction compared with traditional microbiological approaches but noted that many existing studies suffer from limited datasets and lack of clinical validation. Similarly, the systematic review and meta-analysis by Tang et al,[2] reported that machine-learning algorithms generally

achieve high predictive accuracy for AMR, although considerable heterogeneity exists among studies. In the present investigation, the use of a large prospective multicenter cohort and external validation cohort addresses several limitations highlighted in these earlier reports and strengthens the clinical applicability of the developed model.

The superior performance of the multimodal deep neural network compared with logistic regression, Random Forest, and XGBoost demonstrates the importance of integrating multiple data domains. Ali et al,[3] highlighted that future AMR prediction systems should move beyond isolated genomic data and incorporate broader clinical and biological information to improve real-world implementation. Our results support this recommendation, as the multimodal framework significantly outperformed models based on individual data sources alone. The observed increase in predictive performance from clinical-only models to fully integrated models suggests that resistance phenotypes arise from complex interactions between host factors, pathogen genetics, and microbial ecology.

A notable finding of the present study was the strong predictive contribution of genomic resistance determinants, particularly blaNDM and blaCTX-M genes. These genes emerged as the most influential predictors in both multivariable regression and SHAP explainability analyses. Similar observations have been reported by Ren et al,[11] who demonstrated that whole-genome sequencing combined with machine-learning algorithms accurately predicts antimicrobial resistance phenotypes across multiple bacterial species. Likewise, Su et al,[14] concluded that genomic resistance markers represent reliable predictors of phenotypic susceptibility and can potentially shorten the time required for clinical decision-making. Our findings further validate the central role of resistance-associated genetic signatures in AMR prediction.

Metagenomic profiling also contributed substantially to model performance. Reduced microbial diversity was independently associated with resistant infections, and incorporation of metagenomic signatures significantly enhanced predictive discrimination. These findings are in agreement with the work of Van Camp et al,[4] who demonstrated the utility of metagenomic sequencing for identifying pathogens and resistance determinants directly from complex clinical samples. Similarly, Arango-Argoty et al,[8] showed that deep-learning approaches applied to metagenomic data can accurately identify antibiotic resistance genes across diverse microbial communities. The present study extends these observations by integrating metagenomic features with genomic and clinical information within a single predictive framework.

The importance of advanced machine-learning architectures was also evident in our analysis. The deep neural network consistently outperformed conventional machine-learning methods, reflecting its ability to capture complex nonlinear interactions among thousands of variables. Hernàndez-Carnerero et al,[7] reported improved AMR prediction using deep learning and dimensionality reduction strategies, while Asnicar et al,[5] highlighted the growing importance of advanced AI methodologies for microbiome and microbiological data analysis. Our findings support these observations and suggest that deep-learning approaches may be particularly advantageous when handling large-scale multi-omics datasets.

Several previous studies have demonstrated the feasibility of genomic-based resistance prediction in specific bacterial pathogens. Nguyen et al,[9] successfully predicted minimum inhibitory concentrations in nontyphoidal Salmonella using machine-learning approaches and genomic features. Similarly, Moradigaravand et al,[10] reported accurate prediction of antibiotic resistance in Escherichia coli using pan-genome data, while Yang et al,[13] demonstrated successful classification of drug-resistant tuberculosis from sequencing datasets. Compared with these pathogen-specific investigations, our study incorporated multiple bacterial species, clinical risk factors, and metagenomic information, thereby providing a broader and potentially more generalizable framework for AMR prediction.

An important strength of the present study is the use of explainable artificial intelligence techniques. SHAP analysis identified genomic resistance genes, prior antibiotic exposure, microbiome diversity indices, and ICU admission as major contributors to prediction outcomes. These findings align with the recommendations of Drouin et al,[12] who emphasized the importance of interpretable genotype-to-phenotype prediction models for clinical implementation. Explainability remains a critical requirement for clinician acceptance and regulatory approval of AI-based diagnostic systems, and our results demonstrate that high predictive performance can be achieved without sacrificing interpretability.

The clinical implications of this study are considerable. Earlier identification of resistant pathogens could facilitate timely optimization of antimicrobial therapy, reduce inappropriate empirical antibiotic use, and strengthen antimicrobial stewardship programs. As highlighted by Lv and Wang,[6] machine-learning-based AMR prediction has the potential to transform infectious disease management by enabling more personalized treatment strategies. The robust external validation and strong calibration observed in our study suggest that multimodal AI systems may be suitable for deployment in routine clinical settings following further prospective evaluation.

Limitations: Several limitations should be acknowledged. First, although the study was multicentric and prospective, all participating centers were tertiary-care hospitals, which may limit generalizability to community healthcare settings. Second, sequencing and metagenomic analyses require specialized infrastructure and may not be readily available in resource-limited environments. Third, despite external validation, temporal validation in geographically distinct populations was not performed. Finally, the deep-learning framework may require periodic retraining as resistance epidemiology evolves over time.

CONCLUSION

In conclusion, this prospective multicenter study demonstrates that integration of clinical, genomic, and metagenomic data through a multimodal artificial intelligence framework enables highly accurate prediction of antimicrobial resistance. The developed deep-learning model exhibited excellent discrimination, calibration, explainability, and external validity, outperforming conventional machine-learning approaches. These findings support the potential of AI-driven multi-omics platforms to advance precision antimicrobial stewardship and improve the management of infectious diseases in contemporary clinical practice.

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