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Predicting Ampicillin Resistance in Escherichia Coli using an Adaptive Neuro-Fuzzy Inference System (ANFIS): A Proof-of-Concept Computational Approach for Phenotypic Susceptibility Modeling

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ABSTRACT: The increasing prevalence of antimicrobial resistance (AMR) in Escherichia coli threatens the effectiveness of β-lactam antibiotics in both veterinary and human medicine. Conventional susceptibility testing provides categorical interpretations of bacterial response, whereas computational intelligence approaches can model resistance as a continuous and potentially non-linear biological phenomenon. A first-order Takagi-Sugeno Adaptive Neuro-Fuzzy Inference System (ANFIS) was developed to predict ampicillin (AMP, 10 µg) inhibition-zone diameters of bovine E. coli isolates. The model architecture consisted of 78 nodes, 27 fuzzy inference rules, and 54 adjustable parameters. Model training was performed using seven experimentally derived susceptibility observations through a hybrid learning algorithm combining least-squares estimation and gradient descent optimization.The ANFIS model achieved a minimum training Root Mean Square Error (RMSE) of 0.308607, indicating close agreement between observed and predicted values within the training dataset. The predicted inhibition-zone diameter for the evaluated isolate was 11.89 mm, corresponding to a resistant phenotype according to CLSI interpretive criteria (≤ 13 mm). The model successfully generated a continuous susceptibility estimate rather thana simple categorical classification, thereby preserving quantitative information related to bacterial response to ampicillin.The findings demonstrate the feasibility of applying neuro-fuzzy inference systems to antimicrobial susceptibility modeling. Nevertheless, the marked disparity between the number of trainable parameters and available observations (54 versus 7) suggests a substantial risk of overfitting and limits the capacity for biological generalization.Consequently, the present model should be regarded as a proof-of-concept framework requiring validation using larger datasets, cross-validation procedures, and independent external testing before practical deployment.

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1 INTRODUCTION

Antimicrobial resistance (AMR) in Gram-negative pathogens, particularly *Escherichia coli*, represents a major global health challenge at the human-animal-environment interface [?, 5]. The rapid dissemination of resistance determinants against first-line β-lactams—such as ampicillin—jeopardizes empirical therapeutic regimens in both human medicine and veterinary practice [?]. Standard phenotypic susceptibility testing (e.g., Kirby-Bauer disk diffusion) yields continuous zone-of-inhibition measurements that are subsequently categorized into discrete clinical classes: Resistant (R), Intermediate (I), or Susceptible (S) based on standardized thresholds [3]. However, deterministic boundary cutoffs often mask subtle non-linear interactions between bacterial strain dynamics, metabolic fitness costs, and drug diffusion physics [4, 5].

To address these non-linear biological uncertainties, Artificial Intelligence (AI) and Machine Learning (ML) methodologies have been widely explored in predictive microbiology [4,5]. Among these, the **Adaptive Neuro-Fuzzy Inference System (ANFIS)**—originally formulated by Jang [1]—integrates the linguistic interpretability of fuzzy logic systems with the adaptive learning and pattern-recognition capabilities of Artificial Neural Networks (ANNs). ANFIS maps input features to fuzzy membership functions (MFs) and uses a Takagi-Sugeno inference engine [2] to compute continuous quantitative outputs.

The primary objective of this study was to design and evaluate a Takagi-Sugeno ANFIS model [1, 2] for predicting ampicillin (AMP, 10 μg) inhibition-zone diameters in bovine *E. coli* isolates [?], evaluating both its predictive accuracy and structural limitations when constrained by small experimental datasets.

2 MATERIALS AND METHODS

2.1 Experimental Data Source

The empirical dataset was derived from phenotypic disk diffusion assays evaluating *E. coli* susceptibility to ampicillin (10 μg) in cattle isolates from Eastern Algeria [?]. A total of $N = 7$ paired experimental observations (X_i, Y_i) were utilized for model training [?, 1]. The continuous output parameter Y corresponds to the measured zone of inhibition in millimeters (mm).

2.2 ANFIS Architecture and Mathematical Framework

The implemented ANFIS model employs a **first-order Takagi-Sugeno fuzzy inference system** [1, 2]. The network consists of a five-layer feedforward architecture:

1. **Layer 1 (Fuzzification Nodes):** Calculates the membership degrees of input variables using non-linear premise membership functions (e.g., Gaussian MFs) [1]:

$$\mu_{A_i}(x) = \exp\left(-\frac{1}{2}\left(\frac{x - c_i}{a_i}\right)^2\right) \quad (1)$$

Where $\{a_i, c_i\}$ constitute the non-linear premise parameter set [1].

2. **Layer 2 (Rule Nodes):** Computes the firing strength ω_i of each rule using a T-norm product operator [1, 2]:

$$\omega_i = \mu_{A_i}(x_1) \times \mu_{B_i}(x_2) \times \ldots \quad (2)$$

3. **Layer 3 (Normalized Firing Strengths):** Calculates the normalized firing strength $\bar{\omega}_i$ for each rule [1]:

$$\bar{\omega}_i = \frac{\omega_i}{\sum_{j=1}^{R} \omega_j} \quad (3)$$

4. **Layer 4 (Consequent Nodes):** Computes the rule outputs using a linear combination of inputs [2]:

$$f_i = \bar{\omega}_i(p_i x_1 + q_i x_2 + r_i) \quad (4)$$

Where $\{p_i, q_i, r_i\}$ are the linear consequent parameters [1, 2].

5. **Layer 5 (Overall Output Node):** Aggregates the weighted outputs to produce the final continuous prediction [1]:

$$Y_{pred} = \sum_{i=1}^{R} \bar{\omega}_i f_i \quad (5)$$

2.3 Parameter Optimization & Learning Algorithm

Parameter optimization was executed via a **hybrid learning algorithm** [1]:

- **Forward Pass:** Consequent parameters $\{p_i, q_i, r_i\}$ are optimized using the Least Squares Estimator (LSE) [1].
- **Backward Pass:** Premise parameters $\{a_i, c_i\}$ are updated via Gradient Descent (Back-propagation) [1].

Table 1: Structural and Parametric Characteristics of the Formulated ANFIS Model

Structural Parameter	Configuration / Value	Reference
Inference System Type	First-Order Takagi-Sugeno	Takagi & Sugeno (1985) [2]
Total Number of Nodes	78	Model Output [1]
Number of Fuzzy Rules (R)	27	Model Output [1]
Non-linear (Premise) Parameters	27	Jang (1993) [1]
Linear (Consequent) Parameters	27	Jang (1993) [1]
Total Adjustable Parameters (P)	54	Model Output [1]
Checking Sample Pairs	0	Model Output [1]

2.4 Performance Evaluation Metrics

Model accuracy during the learning phase was evaluated using the Root Mean Square Error (RMSE) [1]:

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(Y_{observed,i} - Y_{predicted,i})^2} \quad (6)$$

3 RESULTS

3.1 Training Convergence

The hybrid optimization algorithm converged efficiently across the training epochs [1]. The model achieved a minimum training Root Mean Square Error of **0.308607**. This low residual value indicates that the adaptive network fitted the experimental training observations.

Table 2: ANFIS Training Statistics and Convergence Profile

Metric	Value	Reference / Standard
Final Training Epochs	Convergence reached	Jang (1993) [1]
Minimum Training RMSE	**0.308607**	Model Output
Parameter-to-Sample Ratio (P/N)	$54/7 = \mathbf{7.71}$	Computational Evaluation [1, 6]

3.2 Predictive Phenotype Mapping for Ampicillin

When evaluated for the target *E. coli* isolate under ampicillin (10 μg) exposure, the ANFIS engine generated a continuous output prediction of **11.89 mm**.

To interpret this value biologically, the predicted output was mapped against standardized clinical breakpoints established by CLSI (M100) criteria for Enterobacteriaceae [3]:

- **Resistant (R):** ≤ 13 mm
- **Intermediate (I):** $14 - 16$ mm
- **Susceptible (S):** ≥ 17 mm

Table 3: Phenotypic Classification of Predicted Ampicillin Inhibition Zone

Antibiotic Agent	Disk Content	Predicted Zone	CLSI Breakpoint [3]	Phenotype
Ampicillin (AMP)	10 μg	**11.89 mm**	≤ 13 mm = Resistant	**Resistant (R)**

4 DISCUSSION

4.1 Biological Relevance of the Predicted Resistant Phenotype

The ANFIS-derived prediction of **11.89 mm** places the analyzed isolate within the resistant category according to CLSI interpretive breakpoints (≤ 13 mm) [3]. From a microbiological perspective, this finding is consistent with the widespread occurrence of ampicillin resistance among bovine *E. coli* populations, which is frequently associated with plasmid-mediated β-lactamase production, particularly enzymes belonging to the TEM, SHV, and CTX-M families [**?**]. Rather than producing a binary diagnostic output, ANFIS generated a continuous suscepti-bility estimate. This feature is especially valuable because inhibition-zone diameters inherently represent quantitative biological measurements reflecting multiple interacting processes, in-cluding antibiotic diffusion, bacterial growth kinetics, membrane permeability, and enzymatic degradation of antimicrobial agents [4, 5]. Consequently, continuous prediction may provide a more nuanced description of resistance evolution than traditional categorical classification systems.

4.2 Computational Interpretation of ANFIS Performance

The low training RMSE (0.308607) indicates that the neuro-fuzzy architecture effectively reproduced the training observations [1]. Such performance demonstrates the capacity of ANFIS to model non-linear input-output relationships commonly encountered in microbiological systems, where biological variability and experimental uncertainty frequently violate assumptions of linearity [4].

The predictive behavior of the model can be explained by the combined effects of fuzzy partitioning and adaptive parameter learning [1, 2]. Through the fuzzification process, the

system transforms numerical observations into linguistic representations, while neural-network optimization adjusts membership-function parameters to minimize prediction error [1]. This dual mechanism enables ANFIS to approximate complex decision surfaces even when available information is sparse.

However, excellent training performance should not automatically be interpreted as evidence of predictive reliability [6].

In machine-learning applications, low training error reflects model fit rather than model validity. Therefore, additional performance indicators based on indepen-dent validation data are required before any conclusions regarding predictive accuracy can be established.

4.3 Overfitting Risk and Statistical Limitations

A major limitation of the present study is the disproportionate relationship between model complexity and dataset size [6]. The ANFIS architecture contains 54 adjustable parameters and 27 fuzzy rules, while only seven training observations were available [**?**, 1].

The resulting parameter-to-sample ratio:

$$\frac{P}{N} = \frac{54}{7} = 7.71 \qquad (7)$$

is substantially higher than what is generally recommended in statistical learning theory ($N \gg P$, ideally $N \geq 10 \times P$) [6]. Under such conditions, the model possesses sufficient degrees of freedom to closely reproduce the training data regardless of whether meaningful biological patterns have been learned. Consequently, the obtained RMSE may primarily reflect interpolation of known observations rather than genuine predictive capability.

An additional concern is the absence of a validation dataset. Because no checking or testing data were included during model development ($N_{check} = 0$), the generalization error remains unknown [1]. Therefore, the present findings should be interpreted as preliminary computational evidence rather than confirmation of predictive performance.

4.4 Rule Proliferation and Model Optimization

The generation of 27 fuzzy rules (3^3 grid partitioning) from a dataset containing only seven observations suggests that several rules are likely supported by very limited evidence [1, 7]. This phenomenon, commonly referred to as **rule explosion**, is a well-recognized limitation of grid-partitioning approaches in fuzzy modeling [7].

Future developments should consider data-driven rule-generation techniques such as **Sub-tractive Clustering** [7] or **Fuzzy C-Means (FCM) clustering** [8]. These approaches identify natural structures within the dataset and generate only the rules required to represent underlying data patterns. Reducing the number of rules would decrease model complexity, improve interpretability, and potentially enhance generalization performance [7, 8].

4.5 Future Perspectives for Computational Microbiology

Although preliminary, this work illustrates the potential integration of explainable artificial intelligence approaches within veterinary microbiology [4, 5]. Future studies may expand the ANFIS framework by incorporating molecular and epidemiological predictors, including:

- Presence of specific resistance genes (bla_{TEM}, bla_{SHV}, $bla_{\text{CTX-M}}$) [**?**];
- Minimum inhibitory concentration (MIC) values [3];
- Farm-level antimicrobial usage data and management practices;
- Host age, health status, and clinical history;
- Geographic and temporal surveillance information [5].

The integration of phenotypic, genotypic, and epidemiological variables could transform ANFIS models from simple susceptibility predictors into comprehensive antimicrobial-resistance forecasting systems capable of supporting surveillance and decision-making programs [4, 5].

4.6 Study Limitations

Several limitations must be acknowledged:

Absence of an independent validation or checking dataset ($N_{check} = 0$) [1]; Potential overfitting due to a high parameter-to-observation ratio (≈7.71) [6]; Evaluation restricted to a single antimicrobial agent (Ampicillin, 10 μg) [?];

Lack of direct comparison with alternative machine-learning algorithms such as Random Forests, Support Vector Machines, or Artificial Neural Networks [5].

5 CONCLUSION

This study demonstrates the feasibility of employing a first-order Takagi-Sugeno ANFIS architec-ture to model phenotypic ampicillin resistance in *Escherichia coli* [?]. The system successfully produced a quantitative prediction of inhibition-zone diameter (**11.89 mm**), correctly classifying the isolate as resistant according to established clinical breakpoints. Although the achieved training RMSE (0.308607) suggests strong fitting performance, the limited dataset and high model complexity substantially restrict confidence in predictive generalization. Therefore, the present work should be considered a proof-of-concept investigation illustrating the potential application of neuro-fuzzy systems in computational microbiology. Future research integrating larger datasets, external validation, clustering-based rule reduction, and molecular resistance determinants will be essential to establish the robustness and practical utility of ANFIS-based antimicrobial susceptibility prediction models.

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