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An Ensemble Deep Learning Framework for Single-Cell Phenotypic Profiling of *Escherichia coli* under Antibiotic Stress

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Abstract

Antimicrobial resistance (AMR) is a pressing global health threat, requiring automated and adaptive methods to understand bacterial responses to antibiotics at the single-cell level. In this study, we present a deep learning and ensemble-based framework for morphological profiling and antibiotic classification of *Escherichia coli* using fluorescence microscopic images. Single-cell segmentation is performed with cellpose and auto-generated masks to ensure consistent feature extraction. Thirteen Deep learning architectures, including convolutional networks and vision transformers, are trained to classify untreated cells and those exposed to Chloramphenicol, Mecillinam, MP265, and Nalidixic acid. While the Swin Transformer achieves the highest individual accuracy at 91%, an ensemble combining Swin Transformer, CoaT, and ResNet18 further improves performance. The stacking ensemble provides improved results, with average precision, recall, and F1 scores of 94%. Beyond classification, the framework identifies distinct morphological profiles associated with each antibiotic, including characteristic alterations in cellular geometry, membrane structure, and nucleoid organization. By linking morphological phenotypes to antibiotic mechanisms, this approach establishes an imaging-based methodology for bacterial phenotyping, enables

faster antibiotic screening, and advances the study of antimicrobial resistance through quantitative microscopy.

Keywords: Antimicrobial resistance (AMR); Vision Transformers; *Escherichia coli*; fluorescence microscopy; morphological profiling; stacking ensemble.

1 Introduction

Antibiotics have significantly improved modern medicine by making previously fatal infections manageable and enabling safe surgical procedures, such as chemotherapy and organ transplantation. Additionally, their use continues to spread beyond clinical settings to veterinary medicine and the agricultural industry, especially in countries with low and mid-level income, where they are often used to prevent disease without proper prescription and promote growth in livestock under poorly understood conditions [1]. However, the unchecked overuse of antibiotics has unintentionally increased the development of drug resistance in bacteria. Decades of misuse of drugs have driven bacteria to adapt and evolve, turning once-curable infections into a formidable health threat. As antimicrobial resistance (AMR) spread globally, it threatened to undermine the very foundation of modern medicine. AMR takes place when bacteria have the ability to survive during the exposure of antibiotics that were previously effective against them. As a result, even simple infections such as skin wounds, urinary tract infections, or respiratory illnesses may become challenging to manage.

The consequences of AMR are already severe. In 2019 alone, AMR was directly responsible for an estimated 1.27 million deaths [2–4] and contributed to nearly 4.71 million more in 2021[5], and 400,000 new rifampicin or multidrug-resistant tuberculosis (TB) cases were reported in 2023 [6] with a projection warning of 10 million annual deaths by 2050, exceeding those from cancer[2]. The economic burden is equally severe, with potential losses approaching US\$100 trillion by mid-century. The World Health Organisation (WHO) has identified AMR as one of the top ten global public health threats, anticipating US\$412 billion in annual treatment costs and US\$443 billion in lost productivity by 2035 if left unchecked [3, 5, 8]. Among the most pressing contributors to AMR are multidrug-resistant (MDR) bacteria, many of which belong to a group identified by the WHO as the ESKAPEE pathogens[9, 10]. These organisms employ a range of sophisticated defence mechanisms, including enzymatic degradation of antibiotics, mutations in drug targets, efflux pumps, reduced membrane permeability, and biofilm formation[11, 12]. Inside a bacterial cell, alterations in the nucleoid and ribosomes can signal stress or damage from antibiotic treatment [13–15]. Recent studies have shown that antibiotics can induce rapid and visible phenotypic changes in both Gram-positive and Gram-negative bacteria, including alterations in cell size, shape, and DNA organisation. These changes, if captured accurately, can serve as early indicators of resistance.

Understanding bacterial responses to antibiotic treatment is essential; however, existing diagnostic approaches remain constrained by limited sensitivity and prolonged processing times. Standard antimicrobial susceptibility testing (AST), which typically

relies on growth inhibition assays to determine minimum inhibitory concentrations (MICs), requires 18–24 hours and laboratory infrastructure, limiting its utility in acute settings [16]. Although emerging platforms such as Accelerate’s Pheno System and bioMérieux’s REVEAL Rapid AST have demonstrated improved turnaround times, they remain limited in their ability to resolve phenotypic heterogeneity, detect mixed infections, or identify early morphological responses to antibiotic stress[17, 18]. As a result, broad-spectrum antibiotics are often prescribed empirically, inadvertently accelerating resistance and disrupting the host microbiome. Attempts to accelerate phenotypic AST with single-cell imaging have primarily focused on tracking growth rate or motility across several cell cycles, which is inherently slow and fails to account for persister sub-populations; alternatively, they rely on cytological profiling built on hand-crafted features, a strategy that struggles to generalise across strains[19–21]. Conventional image analysis techniques are similarly labor-intensive and challenging to scale, as they depend extensively on manual annotation and traditional machine learning models. Even where deep-learning (DL) techniques have been adopted, they are used almost exclusively for cell segmentation, because well-curated, openly shared datasets that support higher-level tasks, such as antibiotic-class classification, remain scarce [22].

Despite significant advances in applying deep learning to microbiological imaging for bacterial classification, segmentation, and rapid susceptibility testing [22, 23, 25], current research remains largely diagnostic rather than mechanistic. Existing studies demonstrate impressive accuracy in identifying bacterial species or resistance status, but seldom capture single-cell morphological adaptations or dynamic phenotypic transitions under antibiotic stress. Moreover, most frameworks overlook the mechanistic basis of AMR, focusing instead on binary classification of susceptibility outcomes. This gap highlights a pressing need for integrative approaches that combine high-resolution imaging with advanced computational modeling to elucidate how bacterial morphology and behavior evolve in response to antibiotics.

The present research addresses this gap by utilizing fluorescence microscopy and ensemble DL to characterize antibiotic-induced morphological changes at the single-cell level, thereby advancing from resistance detection toward mechanistic understanding and predictive modeling of bacterial adaptation [26, 27]. To address these limitations, we present an advanced image-based framework for high-throughput, single-cell characterization of *Escherichia coli* exposed to structurally and mechanistically diverse antibiotics. By uniting high-resolution microscopy, DL, and ensemble modeling, our approach addresses critical limitations of current diagnostics and contributes to the broader effort to combat antimicrobial resistance through precise, data-driven characterization of bacterial behavior. The proposed method combines automated segmentation with an ensemble of neural architectures to accurately differentiate cellular responses to distinct antibiotic classes. Beyond improving classification accuracy, the framework enables quantitative analysis of morphological variations in *E. coli*, uncovering antibiotic-specific phenotypic signatures that advance understanding of antimicrobial response mechanisms. This study presents a detailed exposition of the experimental methodology employed, followed by a systematic presentation of the analytical results. It concludes with a comprehensive discussion that interprets the

findings in relation to existing knowledge and their broader theoretical and practical implications.

2 Materials and Methods

The dataset applied in this study was obtained from *DeepBacs: Escherichia coli Antibiotic Phenotyping Object Detection Dataset and YOLOv2 Model* as reported in [28] (available via the Zenodo repository). The dataset comprises microscopy images stored in RGB format, in which each color channel conveys distinct and biologically meaningful information pertinent to the characterization of *Escherichia coli*.

The dataset used antibiotics were used under controlled lab condition to treat *E. coli* cells and their effect were imaged and annotated. Chloramphenicol is used to stop the ribosome and halting protein production and it is used to explore the effects of the cell when it can not make proteins anymore. Mecillinam is applied to inhibit cell wall synthesis and create spherical shapes so that it can be used to study antibiotics affecting morphology. It targets PBP2 a type of protein needed for building the cell wall during growth. MP265 antibiotic target MreB a protein that helps the cell keeping its rod shapes. This antibiotic is used here to change the shape of the cells to study how shape changing drug affect bacterial structure. Lastly, Nalidixate has been used to block DNA replication and see how cells react when they can not duplicate their genetic materials and it targets the enzymes that helps copy DNA.

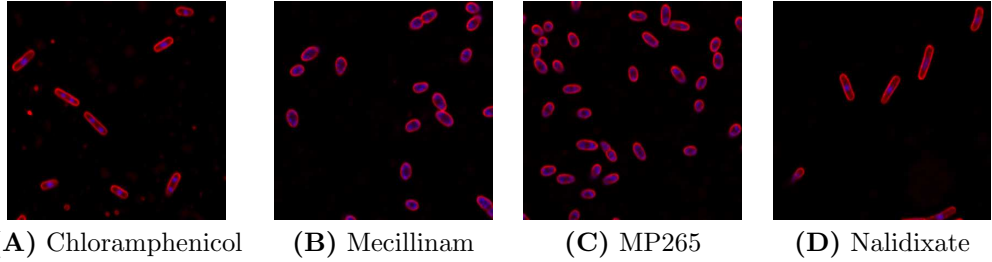


Fig. 1 Representative fluorescence microscopy images of *E. coli* cells treated with four different antibiotics. (A) Chloramphenicol-treated cells show halted protein synthesis. (B) Mecillinam disrupts cell wall synthesis, leading to morphological changes. (C) MP265 interferes with the MreB cytoskeleton, altering cell shape. (D) Nalidixate treatment stalls DNA replication. Membrane (red) and DNA (blue) stains are used to highlight phenotypic differences.

The red channel captures signals associated with the bacterial cell membrane, while the blue channel highlights DNA content. Utilization of these channels enables the differentiation of morphological and structural features through channel-specific variation. The ground-truth annotations employed in this study were generated using ImageJ/Fiji software, with the resulting masks serving as the primary reference for all downstream analyses. This approach ensures a fully automated analysis process and removes the need for manual intervention.

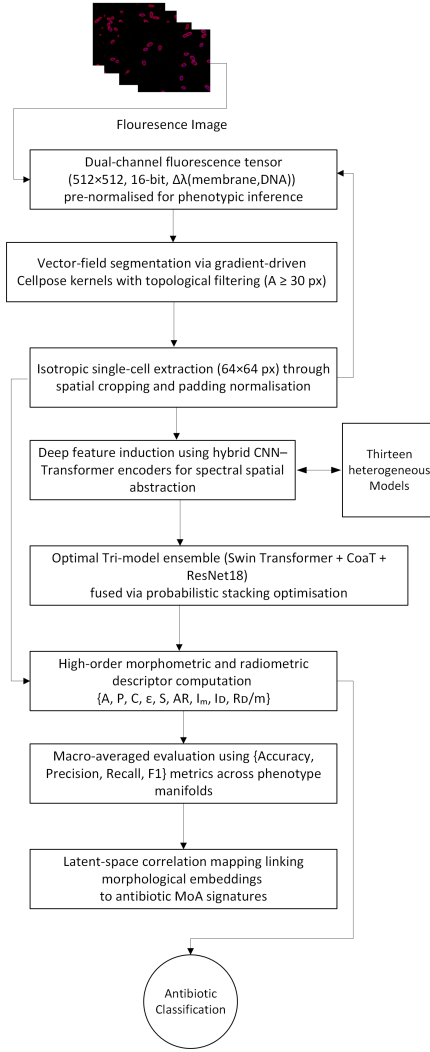


Fig. 2 Overview of the proposed framework for single-cell phenotypic profiling and antibiotic classification of *Escherichia coli*. Dual-channel fluorescence microscopy images containing membrane and DNA signals are processed using Cellpose for automated single-cell segmentation. Small segmented regions (< 30 pixels) are removed, and individual cells are cropped, padded, and resized to 64×64 pixels. The extracted single-cell images are subsequently analysed using 13 CNN- and Transformer-based deep learning architectures for antibiotic-induced phenotype classification. Predictions from Swin Transformer, CoaT, and ResNet18 are further integrated using ensemble learning, with stacking providing the final proposed classification model. In parallel, morphometric and fluorescence-based features describing cellular geometry, membrane structure, and nucleoid organisation are quantified to characterise antibiotic-specific phenotypic responses. Classification performance is evaluated using accuracy, precision, recall, and F1-score, providing an integrated computational framework for antibiotic classification and interpretation of antibiotic-induced morphological changes.

2.1 Automatic Detection of Individual *E. coli* Cells

Segmentation constitutes the foundational operation of this study, as it provides the structural decomposition necessary for single-cell analysis. A pretrained Cellpose model is applied to each microscopy image $\mathbf{I}$, yielding a label field

$$\mathbf{L} = \mathcal{S}(\mathbf{I}), \quad (1)$$

where, $\mathbf{I}$ denotes the input image and $\mathbf{L}$ is the Cellpose-generated label map. Each segmented bacterial cell corresponds to a connected component

$$M_i = \{x \in \Omega \mid \mathbf{L}(x) = i\}, \quad (2)$$

which, defines the set of pixels belonging to the i -th cell. This formulation isolates individual bacterial structures within dense and visually heterogeneous fields, ensuring that subsequent morphological analyses operate on clearly delineated and biologically meaningful regions. The use of multi-channel inputs enables Cellpose to exploit both structural and intensity information for precise delineation of cell boundaries. Small segmented regions containing fewer than 30 pixels were removed to reduce false positives and imaging artifacts. Following segmentation, the predicted instance masks were used to isolate individual bacterial cells, each of which was cropped from the original RGB image, padded to a square, and resized to 64×64 pixels.

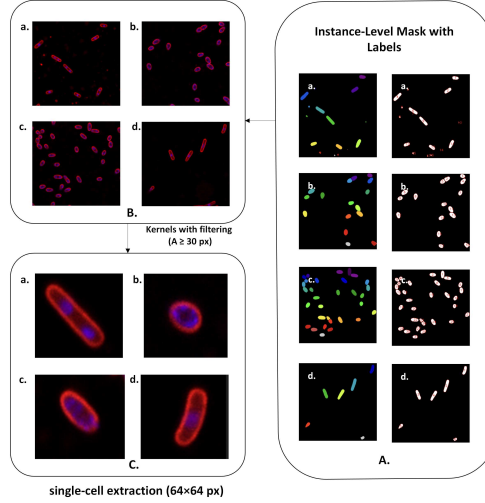


Fig. 3 Fluorescence microscopy images of *Escherichia coli* cells exposed to different antibiotics. Panels (a–d) show representative fields of view (top) and the corresponding images after single-cell extraction (bottom) for: (a) chloramphenicol, (b) mecillinam, (c) MP265, and (d) nalidixic acid. Cell membranes are stained in red and nucleoids in blue, revealing antibiotic-specific morphological and nucleoid alterations.

These patches were saved and labeled according to their antibiotic treatment condition, forming the cell-level dataset for the classification stage.

2.2 Single-Cell Antibiotic Classification Using Fluorescence Microscopy

Classification of *Escherichia coli* cells based on antibiotic-induced morphological alterations is performed to quantitatively assess bacterial responses to different treatments. Fluorescence microscopy images were acquired to visualize both the cell membrane and nucleoid (DNA) structures. Each individual cell was segmented, extracted, and labeled according to its treatment condition: control (no drug), chloramphenicol, mecillinam, MP265, and nalidixic acid. The antibiotics produced distinct morphological phenotypes observable in the membrane and DNA fluorescence channels, including variations such as nucleoid compaction, fragmentation, and membrane deformation. Accurate classification of these phenotypic changes is essential for understanding bacterial adaptation and the mechanisms underlying antibiotic action. Furthermore, automated recognition of single-cell morphological patterns provides a scalable and interpretable framework for profiling bacterial stress responses at the single-cell level.

Let $\mathbf{x}_i \in \mathbb{R}^{H \times W \times 2}$ denote a segmented single-cell fluorescence image and $y_i \in \{1, \dots, 5\}$ its corresponding antibiotic label.

To understand these biologically relevant patterns, we implemented different types of CNNs. VGG16 [34] provides a deep stack of uniform convolutional layers for detecting cell boundaries and nucleoid contrast gradients. ResNet18 [35] utilizes residual learning to capture bacterial structural abnormalities such as elongated membrane morphologies,

$$\mathbf{h}_{l+1} = \mathbf{h}_l + F(\mathbf{h}_l). \quad (3)$$

EfficientNet [36] employs compound scaling to improve performance under limited annotated data. ConNeXt [37] incorporates transformer-inspired optimization with deep convolutional hierarchies and GELU activations to learn features such as cytoplasmic texture and DNA fragmentation. SegNet [38], originally developed for segmentation, preserves spatial hierarchies through its encoder-decoder structure, supporting detection of large-scale morphological variations.

Transformer models embed flattened image patches into vector representations and compute relationships using self-attention. Given patch embeddings $\{\mathbf{z}_j\}_{j=1}^N$, attention is computed as

$$\text{Attention}(Q, K, V) = \text{softmax}\left(\frac{QK^\top}{\sqrt{d}}\right)V. \quad (4)$$

The Vision Transformer (ViT) [39] directly applies this mechanism to image patches to learn structural relationships across membrane and nucleoid signals. Swin Transformer [40] introduces shifted window attention and hierarchical representations, enabling efficient modeling of both local and global morphological variations. MaxViT [41] combines convolutional inductive biases with window-based and axial attention, supporting recognition of complex phenotypes such as nucleoid dispersion involving both local texture and long-range spatial structure.

CNN-based models extract spatially structured patterns through stacked convolutional operations,

$$\mathbf{h}_l = \sigma(\mathbf{W}_l * \mathbf{h}_{l-1}), \quad (5)$$

effectively encoding membrane integrity, curvature, nucleoid compaction, and intensity gradients. However, CNNs are limited in capturing long-range spatial dependencies

under severe morphological stress. Transformer architectures mitigate this limitation by modeling global contextual relationships, enabling improved identification of antibiotic-induced phenotypes such as elongated or fragmented nucleoid structures observed in MP265- and mecillinam-treated cells.

2.3 Meta-Learning Fusion Framework

The classification of *Escherichia coli* cell morphologies under varying antibiotic conditions is a challenging task due to overlapping phenotypic responses, which commonly occur under bacterial stress. To optimize model performance and identify the most effective fusion strategy for enhancing classification accuracy, four ensemble learning approaches were implemented to integrate predictions from three state-of-the-art deep neural networks: Swin Transformer, ResNet18, and CoaT. Each network was independently trained to classify segmented single-cell fluorescence images into five categories: Control, Chloramphenicol, Mecillinam, MP265, and Nalidixic Acid. Morphological variations induced by these antibiotics often exhibit distinct yet subtle structural differences, making it difficult for a single model to achieve consistent classification accuracy. While individual architectures may capture certain morphological cues effectively, they often fail to generalize across all antibiotic-induced patterns. Ensemble learning addresses this limitation by using complementary strengths from multiple architectures, thereby reducing sensitivity to misleading features and improving overall classification.

Four ensemble learning strategies were evaluated to integrate model predictions: hard voting, soft voting, weighted voting, and stacking. Let $\mathcal{M} = \{M_1, M_2, M_3\}$ denote the set of trained models. For an input sample x_i , each model produces a class probability vector

$$\mathbf{p}_i^{(m)} = [p_{i,1}^{(m)}, p_{i,2}^{(m)}, \dots, p_{i,C}^{(m)}], \quad \sum_{c=1}^C p_{i,c}^{(m)} = 1, \quad (6)$$

where, $C = 5$ denotes the number of classes.

In hard voting, each model outputs a predicted label

$$\hat{y}_i^{(m)} = \arg \max_c p_{i,c}^{(m)}, \quad (7)$$

and the final prediction corresponds to the majority class among all models:

$$\hat{y}_i^{\text{hard}} = \text{mode}(\hat{y}_i^{(1)}, \hat{y}_i^{(2)}, \hat{y}_i^{(3)}). \quad (8)$$

Soft voting extends this approach by averaging the predicted probability distributions across models:

$$\bar{\mathbf{p}}_i = \frac{1}{|\mathcal{M}|} \sum_{m=1}^3 \mathbf{p}_i^{(m)}, \quad \hat{y}_i^{\text{soft}} = \arg \max_c \bar{p}_{i,c}. \quad (9)$$

Weighted voting further refines soft voting by assigning a weight w_m to each model based on its validation performance, with $\sum_{m=1}^3 w_m = 1$:

$$\mathbf{p}_i^{\text{weighted}} = \sum_{m=1}^3 w_m \mathbf{p}_i^{(m)}, \quad \hat{y}_i^{\text{weighted}} = \arg \max_c p_{i,c}^{\text{weighted}}. \quad (10)$$

In stacking, the output probabilities from the base models are used as inputs to a meta-classifier. For each sample x_i , the concatenated feature vector is defined as

$$\mathbf{z}_i = [\mathbf{p}_i^{(1)}, \mathbf{p}_i^{(2)}, \mathbf{p}_i^{(3)}] \in \mathbb{R}^{15}, \quad (11)$$

and the final prediction is obtained as

$$\hat{y}_i = f_{\text{meta}}(\mathbf{z}_i), \quad (12)$$

where, f_{meta} denotes the trained meta-classifier.

Among the four approaches, stacking achieved the highest accuracy to predict the class, particularly for borderline morphologies. It notably improved recall for MP265-exposed cells, which often resemble mecillinam-treated cells due to similar elongation patterns but differ in nucleoid texture. Additionally, stacking reduced misclassification of control samples exhibiting normal nucleoid compaction and enhanced the identification accuracy of Nalidixic Acid-treated cells. These results demonstrate that the fusion of heterogeneous deep learning models through stacking effectively addresses subtle phenotypic overlaps and enhances the interpretability of antibiotic-specific morphological signatures.

2.4 Quantitative Morphometric Profiling of Antibiotic-Treated Cells

Antibiotic	Area	Perimeter	Circularity	Eccentricity	Solidity	Aspect Ratio
Control	473.8 $\pm$ 515.3	146.0 $\pm$ 138.2	0.64 $\pm$ 0.40	0.66 $\pm$ 0.36	0.85 $\pm$ 0.07	1.45 $\pm$ 0.35
Antibiotic A	598.8 $\pm$ 745.9	120.1 $\pm$ 127.1	1.02 $\pm$ 0.36	0.67 $\pm$ 0.36	0.88 $\pm$ 0.05	1.38 $\pm$ 0.31
Antibiotic B	427.5 $\pm$ 581.9	113.9 $\pm$ 146.3	1.58 $\pm$ 0.38	0.62 $\pm$ 0.38	0.82 $\pm$ 0.08	1.52 $\pm$ 0.40
Antibiotic C	600.4 $\pm$ 654.4	141.6 $\pm$ 140.7	1.21 $\pm$ 0.34	0.62 $\pm$ 0.34	0.80 $\pm$ 0.09	1.47 $\pm$ 0.38
Antibiotic D	326.6 $\pm$ 455.1	103.1 $\pm$ 129.7	1.66 $\pm$ 0.44	0.61 $\pm$ 0.44	0.83 $\pm$ 0.10	1.55 $\pm$ 0.42

Table 1 Geometric and shape descriptors of cells under different antibiotic treatments.

Antibiotic	Mean Membrane Intensity	Mean DNA Intensity	DNA/Membrane Ratio	Membrane Thickness
Control	101.3 $\pm$ 39.2	66.1 $\pm$ 39.2	0.65 $\pm$ 0.42	4.61 $\pm$ 3.72
Antibiotic A	99.0 $\pm$ 21.0	60.3 $\pm$ 21.0	0.61 $\pm$ 0.63	3.60 $\pm$ 0.34
Antibiotic B	97.8 $\pm$ 19.6	89.5 $\pm$ 19.6	0.95 $\pm$ 0.49	5.01 $\pm$ 0.68
Antibiotic C	100.7 $\pm$ 19.3	83.4 $\pm$ 19.3	0.85 $\pm$ 0.43	5.35 $\pm$ 0.59
Antibiotic D	98.2 $\pm$ 19.6	70.6 $\pm$ 19.6	0.73 $\pm$ 0.73	3.52 $\pm$ 0.18

Table 2 Intensity and structural features of cells under different antibiotic treatments.

Morphological phenotyping using high-resolution fluorescence microscopy offers a quantitative means to examine bacterial cell architecture and subcellular organisation under different antibiotic conditions [42–45]. Multi-channel fluorescence imaging

allows specific cellular components, notably the cell membrane and nucleoid, to be selectively visualised according to their fluorescence signatures. In this study, the membrane and nucleoid were labelled in the red and blue channels, respectively. This arrangement enables accurate segmentation and extraction of morphological descriptors, including cell size and shape, membrane integrity, nucleoid number, and the state of DNA condensation. Such morphometric analysis is essential for characterising phenotypic changes induced by antibiotic exposure, particularly in relation to bacterial stress responses, structural adaptation, and the mechanisms associated with antimicrobial resistance (AMR). The following section outlines the image-based analysis pipeline developed to segment individual cells and quantify their morphological features, thereby supporting systematic comparison of phenotypic variation across antibiotic treatments. Each segmented single-cell image is represented as a two-channel fluorescence patch and treated as an independent sample for downstream analysis. These representations serve as structured inputs for subsequent feature learning and classification models used to distinguish antibiotic-specific morphological phenotypes.

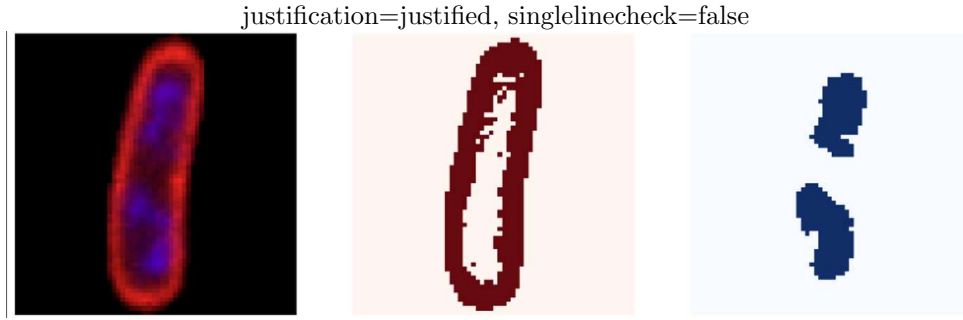


Fig. 4 Extraction of cellular component masks from a fluorescence microscopy image. The original image (left) contains both red (membrane) and blue (nucleoid) fluorescence. From this, separate binary masks are generated: the membrane mask (center) from the red channel and the nucleoid mask (right) from the blue channel. These masks are used for morphological feature analysis.

Cell Shape and Size Changes: The initial analysis concentrates on fundamental geometric and shape descriptors, including cell area, perimeter, circularity, eccentricity, solidity, and aspect ratio. These parameters quantitatively characterize overall cell morphology and are utilized to evaluate the morphological alterations induced by antibiotic, as summarized in Table I.

Antibiotic	Membrane Area	Membrane Perimeter	Circularity	Eccentricity	Solidity	Aspect Ratio
Antibiotic A	1874.85 $\pm$ 567.53	205.92 $\pm$ 46.60	0.59 $\pm$ 0.18	0.77 $\pm$ 0.19	0.93 $\pm$ 0.04	1.88 $\pm$ 0.57
Antibiotic B	1912.32 $\pm$ 415.05	304.03 $\pm$ 60.01	0.30 $\pm$ 0.16	0.72 $\pm$ 0.14	0.85 $\pm$ 0.09	1.59 $\pm$ 0.38
Antibiotic C	2016.32 $\pm$ 493.31	240.82 $\pm$ 61.65	0.51 $\pm$ 0.23	0.72 $\pm$ 0.17	0.92 $\pm$ 0.05	1.65 $\pm$ 0.57
Antibiotic D	1234.47 $\pm$ 414.68	167.05 $\pm$ 23.06	0.55 $\pm$ 0.11	0.92 $\pm$ 0.11	0.94 $\pm$ 0.03	3.14 $\pm$ 0.90
Control	1417.64 $\pm$ 406.75	212.36 $\pm$ 53.15	0.45 $\pm$ 0.19	0.89 $\pm$ 0.10	0.92 $\pm$ 0.05	2.55 $\pm$ 0.69

Table 3 Membrane morphology features per antibiotic treatment group.

Membrane and DNA alterations: The second group of features focuses to fluorescence intensity measurements obtained from the red and blue channels, representing

Antibiotic	Nucleoid Count	Mean Nucleoid Area	Mean Circularity	Mean Solidity
Antibiotic A	0.78 ± 0.42	331.22 ± 223.61	0.72 ± 0.38	0.75 ± 0.40
Antibiotic B	1.15 ± 0.39	1126.53 ± 348.56	0.51 ± 0.17	0.85 ± 0.07
Antibiotic C	1.34 ± 0.50	904.54 ± 450.29	0.59 ± 0.18	0.86 ± 0.09
Antibiotic D	0.94 ± 0.23	250.22 ± 112.12	0.77 ± 0.23	0.88 ± 0.22
Control	1.57 ± 0.64	416.39 ± 292.37	0.61 ± 0.20	0.84 ± 0.16

Table 4 Nucleoid morphology features per antibiotic treatment group.

variations in the cell membrane and nucleoid DNA, respectively (Table II). The mean membrane intensity quantifies the staining level or biophysical properties of the membrane and serves as an indicator of alterations arising from antibiotic-induced modifications in membrane composition or integrity. Similarly, the mean DNA intensity characterizes nucleoid staining, reflecting potential changes in DNA condensation or abundance resulting from antibiotic interference with DNA replication processes. This parameter also provides a normalized metric to assess relative variations between DNA and membrane signals, facilitating the identification of disproportionate nucleoid responses. Such structural modifications may indicate cellular adaptation mechanisms, including membrane reinforcement (thickening) or thinning, aimed at modulating permeability under antibiotic stress.

Membrane Structural Morphology: The third group of features focuses on the quantitative assessment of membrane morphology through parameters such as membrane area, perimeter, circularity, eccentricity, solidity, and aspect ratio (Table III). These descriptors offer critical insights into the geometric configuration and structural integrity of the membrane, enabling differentiation between membrane-specific morphological variations and global cellular shape changes.

Nucleoid Morphology and Organization: The fourth group of features focuses on the nucleoid region, providing essential information about DNA organization and structural integrity (Table IV). Antibiotics that target DNA replication or structure frequently induce pronounced alterations in nucleoid morphology. A markedly reduced nucleoid count may indicate DNA damage, while changes in nucleoid geometry can reflect condensation or expansion processes. To characterize nucleoid shape and compactness, the mean nucleoid circularity and solidity were analyzed. Furthermore, to directly quantify DNA damage associated with AMR, the nucleoid fragmentation index was defined as the variation in nucleoid size or shape within individual cells. This metric captures the degree of DNA fragmentation and structural heterogeneity, often linked to DNA repair activity or stress-response mechanisms contributing to resistance.

3 Results

The evaluation of model performance is crucial for assessing the accuracy and reliability of deep learning (DL) models in classification tasks, particularly in applications such as the precise identification of antibiotics from *E. coli* samples.

The performance comparison: Table V presents detailed evaluation of DL models used for antibiotic-induced phenotype classification in *E. coli* based on single-cell image patches. Among all the models from CNN and Transformer, It is shown that the proposed model perform well than the rest with the highest overall metrics, achieving

94% in accuracy, precision, recall, and F1 score. This shows the model’s capability to extract and interpret subtle morphological changes inside the bacteria cell induced by different antibiotics. Traditional models such as SegNet and VGG16 show significantly lower performance, especially in recall and F1 scores, indicating challenges in capturing the changes of bacterial responses. Transformer-based models, including Swin Transformer, CoaT, and BEiT v2, show strong results, suggesting that attention mechanisms and global context modeling are effective in identifying complex phenotypic patterns in bacterial cells. CNN-based architectures like MobileNetV2, DenseNet121, and ResNet18 also show high scores, indicating that convolutional models still hold relevance when well tuned for the task of classification. The accuracy of models such as EfficientNet and ConvNeXt further highlights the benefits of balanced architecture design that combines feature importance with computational efficiency.

Model	Accuracy (%)	Precision (%)	Recall (%)	F1 Score (%)
SegNet	72.0	75.0	66.0	65.0
VGG16	76.0	80.0	71.0	74.0
BEiT v2	76.0	76.0	75.0	75.0
ViT	77.0	76.0	71.0	72.0
EfficientNet	84.0	83.0	82.0	82.0
MaxViT	84.0	83.0	83.0	83.0
MobileNetV2	87.0	85.0	84.0	84.0
CoaT	88.0	86.0	88.0	86.0
ConvNeXt	88.0	87.0	88.0	87.0
InceptionV3	89.0	87.0	87.0	87.0
DenseNet121	89.0	90.0	87.0	88.0
ResNet18	90.0	91.0	87.0	88.0
Swin Transformer	91.0	91.0	89.0	90.0
Proposed Model	94.0	94.0	93.0	94.0

Table 5 Performance comparison of DL models for antibiotic-induced phenotype classification in *E. coli* using single-cell image patches. Metrics are based on macro-averaged values.

Overall, the comparison between the CNN and Transformer models shows that the combination of well-performing architectures with appropriate preprocessing and effective feature selection plays a crucial role in achieving high-accuracy phenotypic classification in antimicrobial image-based research. Although classification accuracy is often considered the main benchmark for evaluating the performance of deep learning (DL) models, it should not be treated as the only indicator, especially in the context of antibiotic-induced bacterial phenotype classification. Relying solely on accuracy or other basic parameters can be misleading in biological studies where subtle yet biologically meaningful morphological variations are essential for understanding cellular responses. Morphological features such as cell shape, elongation, membrane texture, and nucleoid distribution, as discussed in this study, provide critical biological information that cannot be captured through accuracy metrics alone. These features help explain the mechanism of action (MOA) of antibiotics and distinguish between phenotypes that may appear statistically similar but differ in their biological pathways. Therefore, evaluating DL models should also consider their ability to preserve and

interpret phenotypic characteristics accurately. This ensures that the model not only produces correct predictions but also captures a deeper understanding of bacterial behavior, which is vital for antimicrobial resistance research and drug discovery.

4 Discussion

Using DL Models enables the scope for exploring and understanding the behavior of bacteria towards antibiotics. CNN models and Transformer models are used to classify the type of antibiotic used in the bacteria by understanding the behavioral change in the bacterial cell. The results show several important insights. Firstly, the models were capable of distinguishing different morphological phenotypes based on different antibiotics, like nucleoid induction by Chloramphenicol or cell stretch caused by Mecillinam. However, we do get some misclassification in cases where the morphological cues were less or shared between two types of antibiotic exposure, like elongation patterns, which are common in both Mecillinam and MP265 treatments [50, 51]. Control cells are also sometimes classified as stressed states, leading to false positives in classification. These challenges show that natural differences and complex changes in cell shapes make it hard to classify. For that reason, models need to understand both small details and the overall pattern to identify correctly [52, 53].

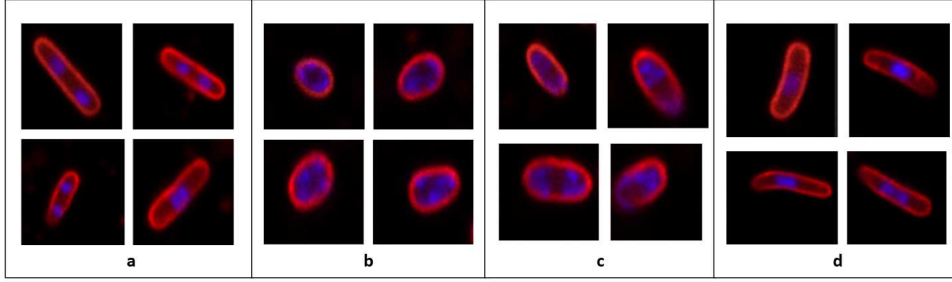


Fig. 5 Fluorescence microscopy images of *E. coli* showing distinct morphological responses under antibiotic treatment: (a) Chloramphenicol, (b) mecillinam, (c) MP265, and (d) nalidixate. Red indicates membrane; blue indicates DNA.

Figure 5, illustrates distinct morphological adaptations of *Escherichia coli* cells under different antibiotic treatments. Chloramphenicol-treated cells (a) maintain a rod-like structure but exhibit condensed nucleoids, reflecting inhibited protein synthesis and halted cellular growth. Exposure to mecillinam (b) results in spherical morphologies due to inhibition of penicillin-binding protein 2, disrupting cell wall synthesis. MP265-treated cells (c) show irregular or bloated shapes consistent with MreB inhibition and loss of cytoskeletal integrity. Nalidixate exposure (d) preserves the rod form but induces elongated nucleoids, reflecting inhibition of DNA gyrase and replication stress. These antibiotic-specific phenotypes confirm that fluorescence microscopy combined with computational analysis can capture subtle yet informative cellular responses, supporting the potential of morphology-based profiling for mechanistic AMR studies.

Our segmentation pipeline is created based on the Cellpose model, which successfully generates high-quality masks for individual cells, which is very important for downstream classification[54]. The main reason for using a segmented mask is to establish a standard that eliminates human intervention throughout the process, thereby enabling the model to operate fully automatically with minimal manual input. This approach helps reduce bias and improve the consistency in analysis. Accurate segmentation reduces the noise from overlapping cells and artifacts in the background, helping the classifier to focus on important and relevant morphological features. The combination of membrane and nucleoid fluorescence channels as model inputs provides a good representation of cellular morphology, enhancing feature extraction [56]. Comparing each model, the transformer-based architecture, such as Swin Transformer, showed good performance likely because of its ability to model long-range spatial dependencies and subtle structural variation. They hybridised CoaT and ResNet18 models, balancing the local feature extraction and global context modelling, which helps to improve the classification due to irregular morphological features and potential sensitivity to image quality [56].

Our framework shows the importance of AI and image-based phenotyping for antimicrobial resistance research. The ability to accurately classify antibiotic treatment at the single-cell level provides a powerful way to understand bacterial responses and guide drug discovery through rapid phenotypic screening. Moreover, Morphological profiling with classification, such as analysing the cell area, eccentricity, and DNA to membrane ratios, adds valuable biological content that can help in understanding mode-of-action (MOA) mechanisms[57, 58]. Understanding antibiotic exposure is the first stepping stone for analysing antimicrobial resistance under the microscope. To understand bacterial behavior, the main obstacle is obtaining sufficient image datasets of single bacteria under increasing antibiotic exposure, which is crucial for understanding the specific resistance mechanisms of bacteria. The automated classification can enhance the field of bacterial image analysis, which can contribute to the study of bacterial growth and behavior regarding AMR[59]. To address the limitations of the proposed transformer-based solution and make it a more generalisable model, the solution can be improved by expanding the dataset, conducting time series analysis, and utilising the third channel as a permeability measure. The dataset used in this study was not particularly created for behavior analysis. We have generated the actual mask to initiate the segmentation, which is crucial for cell analysis. Therefore, a larger dataset with more antibiotic exposure and correct annotation should be collected to improve the result of this study [60].

5 Conclusion

This study introduces a DL-based framework for classifying *Escherichia coli* phenotypes at single-cell resolution under antibiotic stress. By integrating advanced convolutional and transformer architectures with ensemble learning, we achieved high accuracy in distinguishing subtle morphological responses across multiple drug treatments. The inclusion of cell shape descriptors (e.g., circularity, eccentricity, solidity), membrane roughness, nucleoid count, and intensity ratios provided valuable

context for understanding phenotype-specific shifts. The stacking ensemble method outperformed individual models, reflecting the strength of combining local and global features for bacterial classification. Our results show that image-based phenotyping, when paired with AI methods, offers a powerful approach for investigating antibiotic effects at the cellular level. This framework can be extended to support antimicrobial resistance monitoring, phenotype-driven drug discovery, and large-scale screening in microbiological research. In our study, we have used one modality of images, which is fluorescence. In future, the research could be done in other types of images to see the difference and find out which type of images can hold the important features more precisely to understand the mechanism of AMR. To use this classification automated model for antibiotic-based bacteria detection, the trained DL models can be integrated into a real-time classification system with morphological understanding. Making it a user-friendly tool for research use, for quick classification revealing the mechanism of resistance in bacterial cell in emergencies. In addition, this integration can be improved with the feedback from the users.

References

- [1] M. A. Salam *et al.*, “Antimicrobial Resistance: A Growing Serious Threat for Global Public Health,” *Healthcare*, vol. 11, p. 1946, 2023.
- [2] World Health Organization. *Essential Medicines and Health Technologies (EMT), WHO Western Pacific. Health and Economic Impacts of Antimicrobial Resistance in the Western Pacific Region, 2020–2030*. WHO Regional Office for the Western Pacific, 2023.
- [3] V. H. Krishnaprasad and S. Kumar, “Antimicrobial resistance: An ultimate challenge for 21st century scientists, healthcare professionals, and policymakers to save future generations,” *Journal of Medicinal Chemistry*, vol. 67, no. 18, pp. 15927–15930, 2024.
- [4] C. J. Murray *et al.*, “Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis,” *The Lancet*, vol. 399, no. 10325, pp. 629–655, 2022.
- [5] M. Naghavi *et al.*, “Global burden of bacterial antimicrobial resistance 1990–2021,” *The Lancet*, vol. 404, pp. 1199–1226, 2024.
- [6] World Health Organization. *Global Tuberculosis Report 2024*. Geneva, Switzerland: WHO, 2024.
- [7] World Health Organization. Antimicrobial resistance. *Fact Sheets*. Available online: <https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance> (accessed on 6 June 2025).
- [8] Global Leaders Group on AMR. *Recommendations for UN Member States on AMR*. 2024. Available online: <https://www.amrleaders.org/> (accessed on 6 June 2025).

2025).

- [9] N. Oyenuga, J. F. Cobo-Díaz, A. Alvarez-Ordóñez, and E.-A. Alexa, “Overview of antimicrobial resistant ESKAPEE pathogens in food sources and their implications from a One Health perspective,” *Microorganisms*, vol. 12, p. 2084, 2024.
- [10] W. R. Miller and C. A. Arias, “ESKAPE pathogens: Antimicrobial resistance, epidemiology, clinical impact and therapeutics,” *Nature Reviews Microbiology*, vol. 22, pp. 598–616, 2024.
- [11] W. C. Reygaert, “An overview of the antimicrobial resistance mechanisms of bacteria,” *AIMS Microbiology*, vol. 4, pp. 482–501, 2018.
- [12] S. Santajit and N. Indrawattana, “Mechanisms of antimicrobial resistance in ESKAPE pathogens,” *BioMed Research International*, vol. 2016, p. 2475067, 2016.
- [13] H. Yu *et al.*, “Phenotypic antimicrobial susceptibility testing with deep learning video microscopy,” *Analytical Chemistry*, vol. 90, pp. 6314–6322, 2018.
- [14] D. T. Quach, G. Sakoulas, V. Nizet, J. Pogliano, and K. Pogliano, “Bacterial cytological profiling (BCP) as a rapid and accurate antimicrobial susceptibility testing method for *Staphylococcus aureus*,” *EBioMedicine*, vol. 4, pp. 95–103, 2016.
- [15] S. Sridhar, M. Van Dusen, A. Ramesh, P. Yeh, E. Khurana, and R. Chait, “High-content imaging to phenotype antimicrobial effects on individual bacteria at scale,” *mSystems*, vol. 6, p. e00028–21, 2021.
- [16] A. A. Abebe and A. G. Birhanu, “Methicillin resistant *Staphylococcus aureus*: Molecular mechanisms underlying drug resistance development and novel strategies to combat,” *Infection and Drug Resistance*, vol. 16, pp. 7641–7662, 2023.
- [17] Z. A. Khan, M. F. Siddiqui, and S. Park, “Current and emerging methods of antibiotic susceptibility testing,” *Diagnostics*, vol. 9, p. 49, 2019.
- [18] A. Vasala, V. P. Hytönen, and O. H. Laitinen, “Modern tools for rapid diagnostics of antimicrobial resistance,” *Frontiers in Cellular and Infection Microbiology*, 2020.
- [19] V. Kandavalli, P. Karempudi, J. Larsson, and J. Elf, “Rapid antibiotic susceptibility testing and species identification for mixed samples,” *Nature Communications*, vol. 13, pp. 1–8, 2022.
- [20] R. Iriya *et al.*, “Rapid antibiotic susceptibility testing based on bacterial motion patterns with long short-term memory neural networks,” *IEEE Sensors Journal*,

- vol. 20, pp. 4940–4950, 2020.
- [21] H. Yu *et al.*, “Phenotypic antimicrobial susceptibility testing with deep-learning video microscopy,” *Analytical Chemistry*, vol. 90, pp. 6314–6322, 2018.
 - [22] R. Hartmann *et al.*, “Quantitative image analysis of microbial communities with BiofilmQ,” *Nature Microbiology*, vol. 6, pp. 151–156, 2021.
 - [23] X. Wang *et al.*, “A clinical bacterial dataset for deep learning in microbiological rapid on-site evaluation,” *Scientific Data*, vol. 11, p. 608, 2024.
 - [24] A. Signoroni *et al.*, “Hierarchical AI enables global interpretation of culture plates in the era of digital microbiology,” *Nature Communications*, vol. 14, p. 6874, 2023.
 - [25] A. Zagajewski *et al.*, “Deep learning and single-cell phenotyping for rapid antimicrobial susceptibility detection in *Escherichia coli*,” *Communications Biology*, vol. 6, p. 1164, 2023.
 - [26] A. Arnold, S. McLellan, and J. M. Stokes, “How AI can help us beat AMR,” *npj Antimicrobials and Resistance*, vol. 3, p. 18, 2025.
 - [27] B. Ryu, W. Jeon, and D. Kim, “Integrating genomic and molecular data to predict antimicrobial MIC in *Klebsiella pneumoniae*,” *Scientific Reports*, vol. 14, p. 25951, 2024.
 - [28] C. Spahn *et al.*, “DeepBacs for multi-task bacterial image analysis using open-source deep learning approaches,” *Communications Biology*, vol. 5, p. 55, 2022.
 - [29] A. Signoroni *et al.*, “Hierarchical AI enables global interpretation of culture plates in the era of digital microbiology,” *Nature Communications*, vol. 14, p. 6874, 2023.
 - [30] T. Treebupachatsakul and S. Poomrittigul, “Bacteria classification using image processing and deep learning,” in *Proc. 2019 34th Int. Tech. Conf. Circuits/Systems, Computers and Communications (ITC-CSCC)*, pp. 1–3, 2019.
 - [31] D. Quach *et al.*, “Deep learning-driven bacterial cytological profiling to determine antimicrobial mechanisms in *Mycobacterium tuberculosis*,” *Proc. Natl. Acad. Sci. U.S.A.*, vol. 122, no. 6, p. e2419813122, 2025.
 - [32] S. Zoffmann *et al.*, “Machine learning-powered antibiotics phenotypic drug discovery,” *Scientific Reports*, vol. 9, no. 1, p. 5013, 2019.
 - [33] P. Nonejuie, M. Burkart, K. Pogliano, and J. Pogliano, “Bacterial cytological profiling rapidly identifies the cellular pathways targeted by antibacterial molecules,” *Proc. Natl. Acad. Sci. U.S.A.*, vol. 110, no. 40, pp. 16169–16174, 2013.
 - [34] K. Simonyan and A. Zisserman, “Very deep convolutional networks for large-scale image recognition,” arXiv:1409.1556, 2014.

- [35] K. He, X. Zhang, S. Ren, and J. Sun, “Deep residual learning for image recognition,” in *Proc. IEEE Conf. Comput. Vis. Pattern Recognit. (CVPR)*, 2016.
- [36] M. Tan and Q. V. Le, “EfficientNet: Rethinking model scaling for convolutional neural networks,” in *Proc. Int. Conf. Mach. Learn. (ICML)*, 2019.
- [37] Z. Liu, H. Mao, C.-Y. Wu, C. Feichtenhofer, T. Darrell, and S. Xie, “A ConvNet for the 2020s,” in *Proc. IEEE/CVF Conf. Comput. Vis. Pattern Recognit. (CVPR)*, 2022.
- [38] V. Badrinarayanan, A. Kendall, and R. Cipolla, “SegNet: A deep convolutional encoder–decoder architecture for image segmentation,” *IEEE Trans. Pattern Anal. Mach. Intell.*, vol. 39, no. 12, pp. 2481–2495, 2017.
- [39] A. Dosovitskiy *et al.*, “An image is worth 16×16 words: Transformers for image recognition at scale,” arXiv:2010.11929, 2021.
- [40] Z. Liu *et al.*, “Swin Transformer: Hierarchical vision transformer using shifted windows,” in *Proc. IEEE/CVF Int. Conf. Comput. Vis. (ICCV)*, 2021.
- [41] J. Tu *et al.*, “MaxViT: Multi-axis vision transformer,” in *Proc. Eur. Conf. Comput. Vis. (ECCV)*, 2022.
- [42] W. R. Miller and C. A. Arias, “ESKAPE pathogens: Antimicrobial resistance, epidemiology, clinical impact and therapeutics,” *Nature Reviews Microbiology*, vol. 22, pp. 598–616, 2024.
- [43] Z. A. Khan, M. F. Siddiqui, and S. Park, “Current and emerging methods of antibiotic susceptibility testing,” *Diagnostics*, vol. 9, p. 49, 2019.
- [44] A. Vasala, V. P. Hytönen, and O. H. Laitinen, “Modern tools for rapid diagnostics of antimicrobial resistance,” *Frontiers in Cellular and Infection Microbiology*, vol. 10, p. 308, 2020.
- [45] H. Yu *et al.*, “Phenotypic antimicrobial susceptibility testing with deep learning video microscopy,” *Analytical Chemistry*, vol. 90, pp. 6314–6322, 2018.
- [46] M. Kals, E. Kals, J. Kotar, A. Donald, L. Mancini, and P. Cicuta, “Antibiotics change the growth rate heterogeneity and morphology of bacteria,” bioRxiv, 2024.
- [47] A. Tiwari, A. M. Daniels, R. Manley, and F. Gielen, “Harnessing droplet microfluidics and morphology-based deep learning for the label-free study of polymicrobial-phage interactions,” bioRxiv, 2025.
- [48] J. Salgado, J. Rayner, and N. Ojkic, “Advancing antibiotic discovery with bacterial cytological profiling: A high-throughput solution to antimicrobial resistance,” *Frontiers in Microbiology*, vol. 16, p. 1536131, 2025.

- [49] A. Arnold, S. McLellan, and J. M. Stokes, “How AI can help us beat AMR,” *npj Antimicrobials and Resistance*, vol. 3, p. 18, 2025.
- [50] R. Smith *et al.*, “Deep learning recognizes antibiotic modes of action from brightfield microscopy,” *bioRxiv*, 2024.
- [51] E. Hallström, V. Kandavalli, P. Ranefall, J. Elf, and C. Wählby, “Label-free deep learning-based species classification of bacteria imaged by phase-contrast microscopy,” *PLOS Comput. Biol.*, vol. 19, no. 11, p. e1011181, 2023.
- [52] Y. K. Won, C. H. Kim, J. Jeon, J. Cha, and D. H. Lim, “Deep learning by Vision Transformer to classify bacterial and fungal keratitis using different types of anterior segment images,” *Comput. Biol. Med.*, vol. 190, p. 109976, 2025.
- [53] Q. Tang *et al.*, “Morphological profiling for drug discovery in the era of deep learning,” *Brief. Bioinform.*, vol. 25, no. 4, p. bbae284, 2024.
- [54] C. Stringer, T. Wang, M. Michaelos, and M. Pachitariu, “Cellpose: A generalist algorithm for cellular segmentation,” *Nature Methods*, vol. 18, pp. 100–106, 2021.
- [55] X. Zhao, X. Wei, L.-J. Chen, and X.-P. Yan, “Bacterial microenvironment-responsive dual-channel smart imaging-guided on-demand self-regulated photodynamic/chemodynamic synergistic sterilization and wound healing,” *Biomaterials Science*, vol. 10, no. 11, pp. 2907–2916, 2022.
- [56] S. Kotwal *et al.*, “Machine learning and deep learning based hybrid feature extraction and classification model using digital microscopic bacterial images,” *SN Comput. Sci.*, vol. 4, p. 687, 2023.
- [57] H. H. Htoo *et al.*, “Bacterial cytological profiling as a tool to study mechanisms of action of antibiotics that are active against *Acinetobacter baumannii*,” *Antimicrob. Agents Chemother.*, vol. 63, no. 4, p. e02310–18, 2019.
- [58] T. Samernate *et al.*, “High-resolution bacterial cytological profiling reveals intrapopulation morphological variations upon antibiotic exposure,” *Antimicrob. Agents Chemother.*, vol. 67, no. 2, p. e01307–22, 2023.
- [59] S. Lee *et al.*, “Artificial intelligence in bacterial diagnostics and antimicrobial susceptibility testing: Current advances and future prospects,” *Biosens. Bioelectron.*, vol. 280, p. 117399, 2025.
- [60] L. Makrai *et al.*, “Annotated dataset for deep-learning-based bacterial colony detection,” *Scientific Data*, vol. 10, p. 497, 2023.