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Predicting and identifying antimicrobial resistance in the marine environment using AI and machine learning.

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Predicting and Identifying Antimicrobial Resistance in the Marine Environment Using AI and Machine Learning

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***Abstract*—Antimicrobial resistance (AMR) poses a serious threat to public health and serves as a vital reservoir for resistant microorganisms. Antimicrobial resistance (AMR) is an increasingly critical public health issue that requires precise and efficient methodologies to achieve prompt results. The accurate and early detection of AMR is crucial, as its absence can pose life-threatening risks to diverse ecosystems, including the marine environment. This study focuses on evaluating the diameters of the disc diffusion zone and employs Artificial Intelligence (AI) and Machine Learning (ML) techniques such as image segmentation, data augmentation, and deep learning methods to enhance accuracy in predicting microbial resistance.**

***Index Terms*—Artificial intelligence, Machine Learning methods, Inhibition zone measurement, Convolutional Neural Networks, Antimicrobial susceptibility test**

I. Introduction

Antimicrobial resistance (AMR) poses a serious threat to many ecosystem components, including environmental health as well as human and animal populations [1].

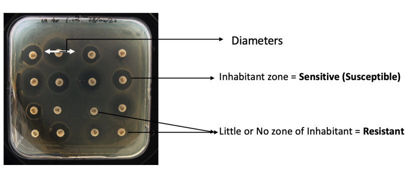

Fig. 1: Key Features Identified in Petri Dish Image

The need for rapid and accurate tools in healthcare is critical, especially for early infection detection and diagnosis of Healthcare-Associated Infections (HAIs). Traditional methods for determining bacterial susceptibility, such as measuring inhibition zone diameters in disc diffusion tests, face challenges due to operator inconsistencies and complex result interpretations. Automating these procedures using segmentation and CNNs can enhance accuracy, reduce manual labour, and speed up the interpretation of antimicrobial susceptibility test results. The disc diffusion method, although widely used for its simplicity and cost-effectiveness, faces several challenges, especially when Inhibition zones are formed around antibiotic discs on the agar plate making this process difficult Fig (2), including overlapping inhibition zones (A) [2], issues related to the seeding of organisms (B), non-homogeneity of the circumference (C), partial efficacy of the antimicrobial (F), and the development of a secondary inhibition zone (D) [3].

Manual measurements as the first choice for laboratory, often taken with rulers, add further complexity. Early detection of infections is crucial, and a proposed solution to improve measuring and examining inhibition zones for determining bacterial antibiotic susceptibility is to develop automatic interpretation methods using ML and AI.

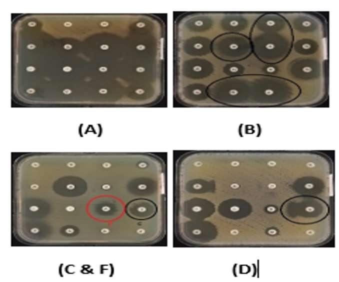

Fig. 2: Difficulty over measurement of inhabitant zone

Recent advancements in computer vision have enhanced AMR prediction capabilities, which is crucial for medical applications and informed decision-making in inpatient treatment. Research has focused on AI, particularly using edge detection and segmentation with Petri dish image datasets, to identify AMR. This study aims to develop automated techniques to calculate inhibition zone diameters around antibiotic discs using advanced ML techniques like U-Net segmentation and the Hough Transform. Our primary contributions in this paper are outlined as follows:

- We used distinct image segmentation approaches, including UNet as a complex model and Hough Transform as a simple classical image processing technique, to detect regions of interest within images.
- Boundary detection techniques such as contour detection were introduced to measure the diameter of inhibition

zones. This involved identifying boundaries with segmentation methods and evaluating parameters like the minimum enclosing circle or distance between points on opposite ends of the zone.
- Deep learning models were developed to categorize bacteria into sensitive, intermediate, and resistant classes based on segmented images. Our findings indicate robust predictive performance from models trained with segmented data.
- Leveraging various Convolutional Neural Networks (CNN), we aim to automate the interpretation of antimicrobial susceptibility tests (AST). These advanced techniques are designed to improve the accuracy, efficiency, and reliability of measuring inhibition zone diameters in disc diffusion methods for bacterial classification.

A. U-Net Method for Inhibition Zone Diameter Measurement

U-Net as a semantic segmentation technique is a CNN architecture commonly used for image segmentation tasks and is the most famous end-to-end Fully Convolutional Network(FCN) model, which is known for its ability to effectively segment objects in biomedical images [4]. Its distinctive U-shaped design comprises an encoder and decoder network connected through skip connections. The encoder part of the U-Net architecture uses successive Convolutional and pooling layers to extract high-level features from the input image [7]. The U-Net architecture can segment the inhibition zones accurately by learning from labelled training data. Image Pre-processing: This method like all other segmentation methods starts with image acquisition and pre-processing to enhance the image quality. Normalize the pixel values to a standard range (e.g., [0, 1])

$$f_{\text{norm}}(x,y) = \frac{f(x,y) - f_{\min}}{f_{\max} - f_{\min}} \tag{1}$$

to find the minimum and maximum pixel values in the image, respectively. U-Net Architecture be formed of an encoder and a decoder, where the Encoder Path applies convolutional layers followed by max-pooling layers to downsample the input image, capturing context and reducing spatial dimensions based on the below mathematical equation : Convolutional layer: where **W** is the filter, denotes the convolution operation, and **b** is the bias

$$I_{\text{conv}} = W * I + b \tag{2}$$

Max-pooling layer:

$$I_{\text{pool}}(x,y) = \max_{i,j \in [0,k-1]} I_{\text{conv}}(x+i, y+j) \tag{3}$$

where **k** is the size of the pooling window.

Decoder Path: this path applies transposed convolutions (upsampling) to increase the spatial dimensions and combines features from the encoder via skip connections to ensure precise localization.Transposed convolution:

$$I_{\text{up}} = W_{\text{up}} * I_{\text{pool}} + b_{\text{up}} \tag{4}$$

where I_{up} is the upsampled output, W_{up} is the transposed convolution filter, I_{pool} is the input feature map to be upsampled, and b_{up} is the bias. Concatenation with encoder features:

$$I_{\text{concat}} = [I_{\text{up}}, I_{\text{encoder}}] \tag{5}$$

Output Layer: The final layer applies a softmax activation to produce a probability map for each class (e.g., inhibition zone vs. background). Softmax activation:

$$P(c|x,y) = \frac{e^{Z_c(x,y)}}{\sum_{c'} e^{Z_{c'}(x,y)}} \tag{6}$$

Segmentation and Diameter Measurement: After training the U-Net on labelled data, it can segment inhibition zones in new images. The U-Net outputs a binary mask where inhibition zones are labelled, which shows the Segmentation Result

$$\text{Mask}(x,y) = \begin{cases} 1 & \text{if pixel } (x,y) \text{ belongs to the inhibition zone} \\ 0 & \text{otherwise} \end{cases}$$

Diameter calculation: Measure the diameter of the segmented inhibition zones. This can be done by finding the bounding box of the connected components in the binary mask and calculating the diameter

$$D = \sqrt{(x_{\max} - x_{\min})^2 + (y_{\max} - y_{\min})^2} \tag{7}$$

Conversion to physical dimensions: Convert the diameter from pixels to physical units using the known scale.

$$D_{\text{physical}} = D \times \text{scale} \tag{8}$$

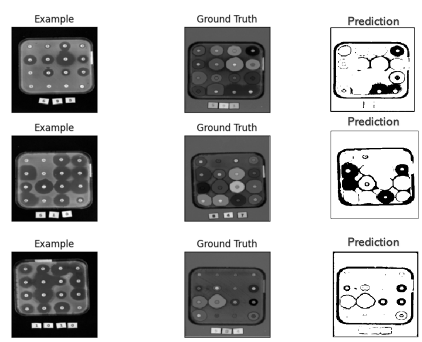

Fig. 3: U-Net Architecture Prediction for Inhibition Zone Segmentation

B. Hough Transform for Circle Detection in Inhibition Zone Diameter Measurement

Another feature extraction method used in image processing, analysis and computer vision is the Hough Transform. It is used for detecting basic shapes in the image like ellipses, circles, and lines [5]. In this method, Pre-processing is carried out to improve image quality and applicability, before image acquisition, much like in watershed segmentation, Pre-processing techniques that are frequently used are grayscale conversion, contrast enhancement, and noise reduction. Then the diameter is measured by the Edge Recognition technique with the Canny algorithm to find the edges in the image before the Hough Transform is applied. The Canny edge detection algorithm is commonly used for this purpose. After identifying the edges in the image, we will Apply the Hough Transform for Circle Detection, this method works by mapping edge points into the parameter space of circles and finding the most likely circle parameters (centre and radius). The detected circles are represented by their centres (x, y) and radii (r). The diameter **D** of each circle is simply twice the radius for Calculating Diameter [5]. The step-by-step procedure to measure the zone of inhibition using this method is shown in the flowchart below:

Input Image → Image Pre-Processing → Image Enhancement → Canny Edge detection → Hough Transform → Calculating Diameter

Fig. 4: Sequential Steps of procedure to measure the zone of inhibition using Hough Transform method

The Edge Recognition method is given by the following mathematical form:

Edge Detection: The gradient magnitude $M(x, y)$ and direction $\alpha(x, y)$ are given by:

$$M(x, y) = \sqrt{G_x^2 + G_y^2} \tag{9}$$

$$\alpha(x, y) = \tan^{-1}\left(\frac{G_y}{G_x}\right) \tag{10}$$

where G_x and G_y are the gradients in the x and y directions, respectively. Hough Circle Transform [5] is simplified to be stored in a two-based structure, the first step Accumulator Array, in which each edge pixel and circle are drawn in the parameter space, and the accumulator array is used to detect the best candidate circles. and the second step is Parameter Space, The parameters are (a, b, r), where (a, b) is the centre and r is the radius of the circle. The parametric equation of a circle is:

$$(x - a)^2 + (y - b)^2 = r^2 \tag{11}$$

For each edge point (x_i, y_i) detected by the Canny algorithm, the potential circle centres (a, b) are computed for a range of radii r. The accumulator array $A(a, b, r)$ is incremented for each candidate circle:

$$A(a, b, r) = A(a, b, r) + 1 \tag{12}$$

This process can be expressed as:

$$\begin{aligned} &\text{For each edge point } (x_i, y_i): \\ &\quad \text{For each possible radius } r: \\ &\quad\quad \text{For each possible angle } \theta: \\ &\quad\quad\quad a = x_i - r\cos(\theta) \\ &\quad\quad\quad b = y_i - r\sin(\theta) \\ &\quad\quad\quad A(a, b, r) = A(a, b, r) + 1 \end{aligned} \tag{13}$$

The circles are detected by finding local maxima in the accumulator array. The center (a, b) and radius r corresponding to the maxima are considered as detected circles:

$$(a_k, b_k, r_k) \text{ where } A(a_k, b_k, r_k) \text{is a local max} \tag{14}$$

Diameter Calculation: The diameter is twice the radius obtained from the Hough Transform. So, the diameter D of each detected circle is twice the radius: D = 2r

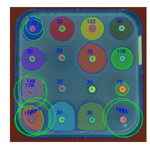

Fig. 5: Measurement of diameter with Hough Transform Architecture for Inhibition Zone

II. Materials & Methods

This section presents two distinct image segmentation methods—U-Net, a deep learning architecture, and the Hough Transform, a classical image processing technique. The study also introduces boundary detection techniques to measure inhibition zone diameters and categorizes bacteria into three classes: sensitive, intermediate, and resistant. By using publicly available datasets in [6]. The data collected for training segmentation approaches and deep learning models were carefully gathered. Different methods were evaluated for accuracy, advantages, and drawbacks. Models used images of an inhibition zone, indicating the automatically measured diameter and detected antibiotic with a dashed circle.and also evaluated for their ability to distinguish between classes, and their results were compared.

III. Datasets

In this study, we consider publicly available datasets, which are available online at http://stat.genopole.cnrs.fr/ast.zip. These datasets consist of images of Petri dishes with inhibition zones, used for training segmentation approaches and deep learning models. The protocol for gathering these datasets has been carefully considered [6].

IV. Processing

Figure 6 illustrates the image processing pipeline for annotating Petri dish images. Maintaining consistent image brightness and contrast are crucial, although variations can occur due to equipment differences and image quality issues, such as broken plates or low contrast between bacteria and inhibition zones [6]. Histogram equalization was applied to enhance consistency across all images in the dataset, addressing lower contrast areas typically found in problematic images. All AST plates were captured using a smartphone camera(Honor 6x, 2 megapixels), ensuring uniform image quality essential for minimizing noise and optimizing feature recognition by the model, thereby improving overall performance.

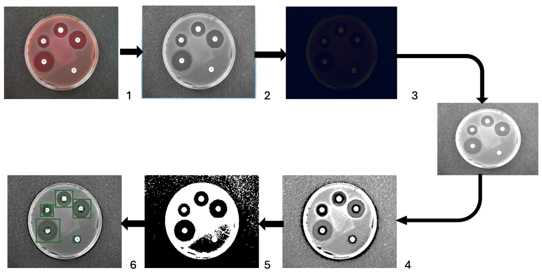

Fig. 6: Application of Annotation to Segmentation in a Petri Dish Image Sample: (1) Median Filter; (2) Detect Edges; (3) Binarize Image; (4) Fill holes; (5) Erode Image; (6) Segmentation & Bound Boxing.

V. Methodology

This study explores various segmentation techniques using CNNs [8] to automate inhibition zone detection, aiming to improve precision and efficiency in measuring zone diameters and classifying bacteria. A dataset of 253 petri dish images and 8,349 images of pills on dishes was used for training. Images were selected based on clarity, the presence of inhibition zones, and quality. Preprocessing steps included resizing, normalization, and augmentation like scaling for improved generalization. The UNet architecture in PyTorch was utilized, featuring a contracting path for context capture, a bottleneck for processing, and an expansive path for localization through upsampling and concatenation. Another method employed is the Hough Transform, specifically for detecting and measuring inhibition zones in disc diffusion assays using OpenCV in the training process. Each model was trained on the collected dataset with the following configurations: Training dataset split: 80% training, 20% validation. Number of epochs: 50, Batch size: 16, Learning rate: 0.001 for UNet. The following metrics—accuracy, inter-section over union (IoU), and dice coefficient—were used to evaluate the performance of the models. The results of the model were compared to determine the most effective approach for segmenting inhibition zones. A summary of the performance metrics is presented in Table(I).

- Intersection over Union (IoU) evaluates the overlap between the predicted bounding box and the ground truth bounding box:

$$\text{IoU} = \frac{\text{Area of Overlap}}{\text{Area of Union}} \tag{15}$$

- Dice Coefficient (F1 Score) is calculated as:

$$\text{F1 Score} = \frac{2 \times \text{Area of Overlap}}{\text{Total Area of Predicted} + \text{Total Area of Ground Truth}} \tag{16}$$

The models were evaluated using accuracy, Intersection over Union (IoU), and Dice Coefficient metrics. The U-Net model demonstrated superior performance in all categories compared to the Hough Transform method.

TABLE I: Performance Comparison of Segmentation Models

Model	Accuracy	IoU	Dice Coefficient
UNet	0.95	0.92	0.91
Hough Transform	0.89	0.85	0.84

The results suggest that deep learning models, particularly CNNs, are highly effective for automating the measurement of inhibition zones.

VI. Conclusion and Future scope

In this study, we introduce an advanced deep learning-based method for accurately measuring disc diffusion zone diameters in antimicrobial susceptibility testing (AST). Our approach aims to enhance AI and ML capabilities to improve patient outcomes and global efforts against antimicrobial resistance. By leveraging CNN-based segmentation models, we demonstrate effective measurement of inhibition zone diameters and aim to expand datasets from human and animal populations to refine resistance patterns. This initiative seeks to advance AMR understanding and management, benefiting public health and clinical outcomes. Future work will focus on enhancing model performance, expanding datasets to improve AMR detection in diverse environments, and exploring broader biomedical image analysis applications.

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