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*Original Article*

# Disc Diffusion Reader: an AI-powered potential solution to combat antibiotic resistance in developing countries

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## Abstract

Introduction: Antimicrobial resistance (AMR) is a global health challenge, and antimicrobial susceptibility testing (AST) is vital for guiding treatment. Although widely used, the Kirby-Bauer method depends on skilled interpretation, which can be time-intensive and error-prone. This study explored the potential of an artificial intelligence (AI)-driven progressive web app (PWA) to automate the analysis of Kirby-Bauer test images, thereby enhancing accuracy and efficiency.

Methodology: Images of Kirby-Bauer test results were annotated to train the Faster R-CNN ResNet-50 to detect agar plates, inhibition zones, and antibiotic discs. MobileNetv2 was used for antibiotic disc classification. A Human-in-the-Loop (HITL) approach enabled technicians to correct errors and improve model performance through retraining. The PWA, built with VueJS and Python-PHP, provided real-time analysis aligned with the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards.

Results: The application achieved 92.95% accuracy for inhibition zone detection and 96.92% accuracy for antibiotic disc identification, with a performance improvement of 99.28% following HITL corrections. The measurements closely aligned with those of the technicians in 89.54% of the cases. The system processed up to 50 images per hour, supporting reliable and rapid AST workflow.

Conclusions: The AI-powered "Disc Diffusion Reader" demonstrated high accuracy and efficiency, by reducing interpretation variability in the AST workflows. Its scalability and adaptability, particularly in low-resource settings, make it a valuable tool for combating AMR. Continuous retraining and validation will ensure sustained reliability, and highlight the potential of AI-driven solutions in modern microbiology.

**Key words:** drug resistance; artificial intelligence; developing countries; disc diffusion method; neural networks.

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## Introduction

The emergence of antimicrobial resistance (AMR) has been the most significant challenge to global public health, especially in low- and middle-income countries. AMR surveillance in these countries is limited by poorly functioning health systems, financial resources, and lack of skilled personnel and data management systems [1,2]. Antibiogram methods are being improved to optimize the treatment duration and monitor AMR [3]. However, previous research indicates that the agar disc diffusion method (Kirby–Bauer method) is still the most common method employed by microbiology laboratories in developing countries, such as countries in Africa and Asian low- and middle-income countries [4,5].

The Kirby-Bauer method provides several advantages, such as simplicity, flexibility in selecting antimicrobials, and cost-effectiveness. These features make it one of the most widely accepted and commonly used methods, particularly in laboratories with low-to-medium throughput. The Kirby-Bauer method is a time-consuming, multi-step analytical process that relies on technicians' expertise and has many complex interpretation rules. Therefore, it is necessary to have the support of artificial intelligence (AI)-based tools to reduce variability due to operator manipulation and interpretation, thus shortening the time to return the results to the clinic. [6,7].

AI can potentially revolutionize the analysis of Kirby-Bauer test results. By employing image recognition and deep learning, AI can automate the traditional labor-intensive process of measuring inhibition zones, detecting antibiotic labels, reducing human error, and enhancing reproducibility. The systems can be trained to identify bacterial resistance patterns accurately and provide rapid and reliable susceptibility profiles. Ultimately, AI-driven analysis can streamline clinical workflow, and enable faster and more informed antibiotic treatment decisions. [8,9].

In this study, we developed a progressive web app (PWA) integrated with AI models using computer vision to address the challenge of determining parameters in antibiotic susceptibility test (AST) result interpretation. This tool promises to help technicians by streamlining data organization, reducing the time required for analysis, and enhancing the overall performance of the Kirby-Bauer test.

## Methodology

*Dataset and training AI model for object detection*

Images of Kirby-Bauer AST results were collected from the Department of Microbiology at Hue University of Medicine and Pharmacy Hospital. The standardized imaging protocols were followed to ensure consistent and high-quality image capture. The images were captured using digital cameras with a minimum resolution of 1920 × 1440 pixels, positioned approximately 20 cm above the agar plates using a stable stand to maintain consistent distance and focus. Uniform LED ring illumination was employed to provide even lighting and minimize shadows, which is crucial for accurately interpreting inhibition zones. Additionally, a capture-box overlay was integrated within the imaging software to assist users in aligning and framing the agar plates correctly, ensuring that images are centered and uniformly scaled. These standardized protocols aimed to enhance reproducibility and accuracy across different laboratory settings.

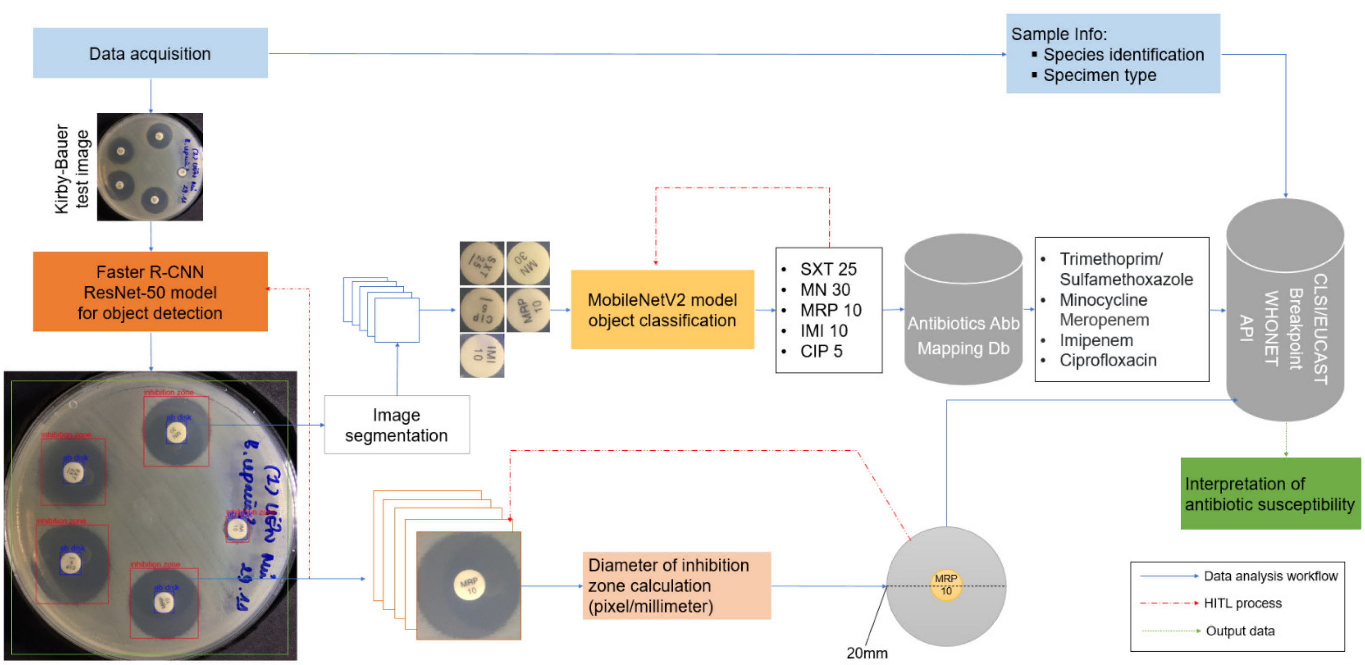
LabelImg, an open-source tool, to label images to train a deep-learning-based object detection model, was used for annotation. Each plate image was annotated with three objects: the agar plate (labeled as "plate"), the inhibition zone (labeled as "ib_zone"), and the antibiotic disc (labeled as "ab_disc") [10]. The Faster R-CNN ResNet-50 model was used for object detection on Kirby-Bauer test images [11,12]. The Faster R-CNN architecture was selected for its effective two-stage object detection process, with ResNet-50 serving as the backbone for feature extraction. The model was initialized with pre-trained weights and fine-tuned on our custom dataset of labeled Kirby-Bauer test plates.

The Azure Machine Learning cloud platform was used to train the model on an antibiotic susceptibility dataset. Training and evaluation were conducted on a Linux operating system with a 6-core vCPU, an NVIDIA Tesla V100 16GB GPU, 112 GB of RAM, and 336 GB of disc space (Microsoft, Redmond, WA, USA). The model was developed in Python 3.8.10, utilizing the PyTorch-Ignite 0.4.12 framework [13].

*Object classification for determining antibiotics abbreviation discs*

Antibiotic abbreviation discs are the letters on the discs that indicate the identity and concentration (in

**Figure 1.** Methodology of the research study.




API: application programming interface; CIP: ciprofloxacin; CLSI: Clinical and Laboratory Standards Institute; Db: database; HITL: human-in-the-loop; IMI: imipenem; MRP: meropenem; MN: ninocycline; SXT: trimethoprim/sulfamethoxazole; WHONET: World Health Organization Network for Surveillance of Antimicrobial Resistance micrograms) of the antibiotic in the disc. The Faster R-CNN model, a well-known architecture for object detection, was employed to identify the zones of inhibition and antibiotic abbreviation discs within the uploaded AST result images. This model generated bounding boxes around the detected objects and precisely localized these key features. Once the antibiotic discs were detected, their images were cropped and sent to the classification model, MobileNetv2 [14]. This model, known for its efficiency and accuracy in image classification, was trained to identify the specific antibiotic disc abbreviations in the extracted text on the disc images (Figure 1).

The antibiotic paper disc was a reference scale for converting pixel measurements into millimeters. The AI model first identified the bounding box of the antibiotic disc and calculated its diameter in pixels, using the known actual diameter of 6 mm as a calibration standard. Simultaneously, the model detected the inhibition zone, which appeared as two concentric circles and determined its diameter in pixels. Using this reference, a conversion formula was applied to accurately translate the inhibition zone's diameter from pixels to millimeters. The algorithm automatically converted the pixel measurements to millimeters using the formula

$$D_{\text{ib\_zone}} = \frac{d_{\text{ab\_disc}} \times r_{\text{ab\_disc}}}{R_{\text{ib\_zone}}}$$

where: $D_{\text{ib\_zone}}$, and $d_{\text{ab\_disc}}$ were in millimeters, and $r_{\text{ab\_disc}}$ and $R_{\text{ib\_zone}}$ were in pixels.

*Integrated Human-in-the-Loop (HITL) and fine-tuning*

Integrating HITL and fine-tuning approaches into the AI system for analyzing Kirby-Bauer test results can significantly enhance the model's accuracy and adaptability, especially in real-world variable laboratory conditions [15,16]. The proposed system integrates HITL feedback with continuous fine-tuning to enhance the AI-based analysis of Kirby-Bauer test results. Technicians started by uploading photos of the test plates, where the AI model automatically detected inhibition zones and measured their diameters. These preliminary results were presented in an interactive interface, allowing the technicians to adjust the zone boundaries using draggable and resizable elements if any inaccuracies were identified. The adjusted measurements were then saved, storing the AI predictions and technician corrections in a structured database tagged as "corrected" data.

The system was designed to periodically initiate a fine-tuning process using newly corrected feedback data, ensuring continuous improvement of the AI model without disrupting ongoing operations. Specific mechanisms were integrated to trigger manual intervention when necessary to enhance accuracy and reliability. Objects such as inhibition zones and antibiotic discs with confidence scores below 90% were flagged for human verification. Additionally, zone diameters that deviated by more than 2 mm from the AI's initial prediction required manual validation, and antibiotic labels absent from the database prompted human input for correction and future inclusion. To maintain adaptability, the model underwent automated retraining weekly or when at least 50 corrected cases had accumulated. This retraining process was executed via a cloud-based pipeline, allowing seamless integration of updates without disrupting real-time operations. Once the fine-tuning process was complete, the updated model was automatically deployed, incorporating the latest corrections and enhancing performance.

*PWA and AI-powered model deployment*

The Kirby-Bauer analysis system was implemented as a PWA combined with an AI-powered backend for automated test interpretation, to enhance accessibility and streamline user interaction. This approach ensured that the platform was scalable and easily accessible across various devices, including desktops, tablets, and mobile phones; without needing dedicated native applications.

The "Disc Diffusion Reader" PWA was developed using a combination of frontend and backend components. The front end, designed using VueJS, provided a user-friendly interface for uploading AST images, performing image preprocessing, and visualizing the final results. This interactive interface allowed users to select and adjust image parameters to ensure optimal visual representation of the antibiogram data. The application's backend relied on a combination of PHP and Python services. PHP was utilized to create API endpoints that handled image uploads and data transfers between frontend and backend systems [17]. Python, in contrast, powered core image analysis processes. This included implementing the Faster R-CNN object detection model to identify inhibition zones and antibiotic discs, and the MobileNetv2 classification model for determining the antibiotic name abbreviation on the disc and calculating the diameter of inhibition zones. By integrating frontend and backend functionalities, this modular design enabled a streamlined and efficient workflow for automated AST analysis on cross-devices.

**Table 1.** Object detection performance of Faster R-CNN ResNet-50 model on Kirby-Bauer test images.

| Metric | Description | Value |
|---|---|---|
| Total images in the dataset | Number of Kirby-Bauer images analyzed | 211 |
| Bacterial groups included | Types of bacteria included in the dataset | *E. coli, S. aureus, P. aeruginosa, K. pneumoniae* |
| Objects detected | Types of objects identified | Test plate, antibiotic discs, inhibition zones |
| Plate size | Diameter of Kirby-Bauer test plates | 90 mm |
| Antibiotic discs per plate | Range of antibiotic discs per plate | 4–6 discs |
| Prediction time | Average time taken per image for object detection | 0.42–0.84 seconds/image |
| Confidence score range | Detection confidence scores achieved by the model | 90.0–99.9% |
| Images with low confidence (< 90%) | Percentage of images with at least one object < 90% score | 5.2% (11 of 211 images) |
| Objects with a low confidence score | Types of objects with scores < 90% | Inhibition zone (dominant), antibiotic disc |

The application incorporated comprehensive laboratory information management functions, including user registration, login, and Kirby-Bauer antibiotic test image data management. The image data included specimen types, identified bacterial strains, taken date, and patient ID. These features enabled the efficient tracking and organization of laboratory data. All information was securely stored in a MySQL database, ensuring robust data management and facilitating easy retrieval for further analysis and reporting [17]. An antibiotic mapping database was established by storing information on 151 antibiotics in the MySQL database. The stored data included WHONet Abx codes, Clinical and Laboratory Standards Institute (CLSI M100, 34$^{th}$ edition) and European Committee on Antimicrobial Susceptibility Testing (EUCAST version 13.0, 2023) guidelines, commercial abbreviations, antibiotic names, and drug classes (Figure 2).

*Evaluating the performance of an AI-powered app vs. technicians in the AST Kirby-Bauer test*

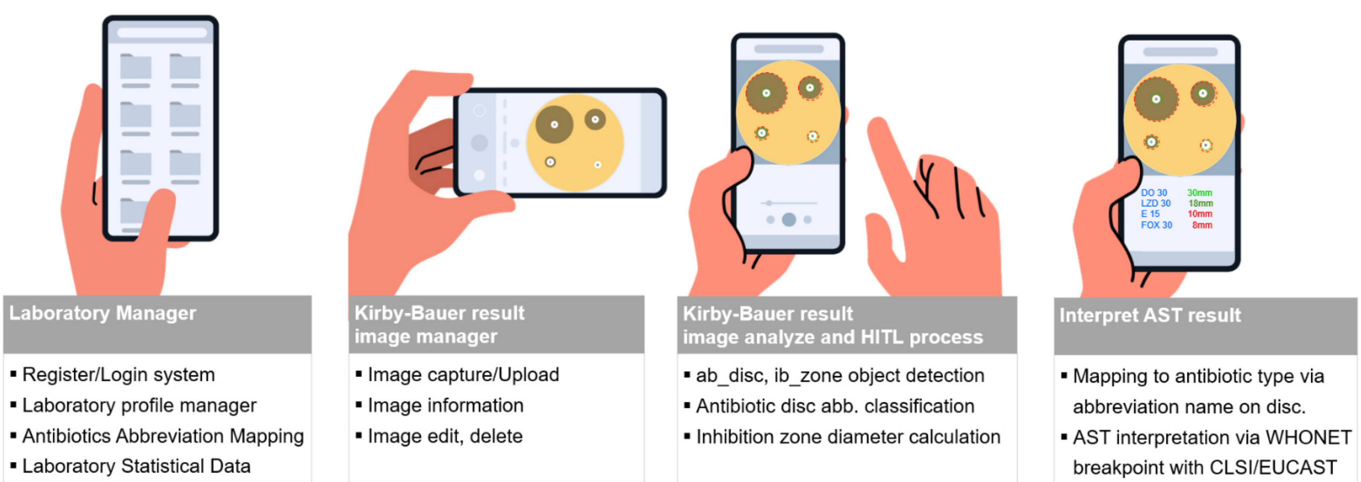
A dataset of 211 Kirby-Bauer disc diffusion test result images was utilized to evaluate the performance of the AI-powered application in analyzing and interpreting AST. This comparison was used to assess the app's accuracy, speed, and consistency with trained laboratory technicians. The potential of AI in enhancing efficiency and reducing human errors in AST analysis was demonstrated by assessing critical metrics, such as antibiotic classification, inhibition zone diameter measurement, susceptibility categorization, and interpretation against CLSI standards. This comparison provided valuable insights into the reliability and practicality of integrating AI technology into routine laboratory workflow.

## Results

*Object detection by AI model on Kirby-Bauer test result image*

The Faster R-CNN ResNet-50 model was successfully employed to detect 3 key objects in the Kirby-Bauer test result images: test plate, antibiotic discs, and inhibition zones. The model was tested on a dataset of 211 Kirby-Bauer images covering 4 common bacterial groups: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. The Kirby-Bauer test used a 90 mm plate containing 4 to 6 antibiotic discs. The AI model achieved confidence scores ranging from 90.0% to

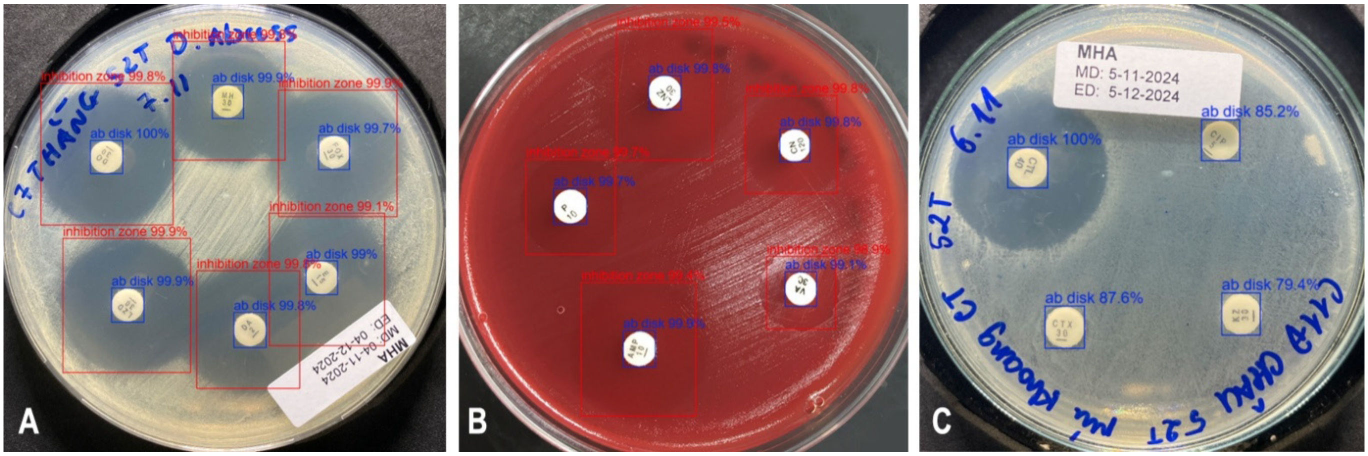
**Figure 2.** Analysis of an antibiotic susceptibility test (AST) plate workflow with the "Disc Diffusion Reader" app.




HITL: human-in-the-loop. CLSI: Clinical and Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing

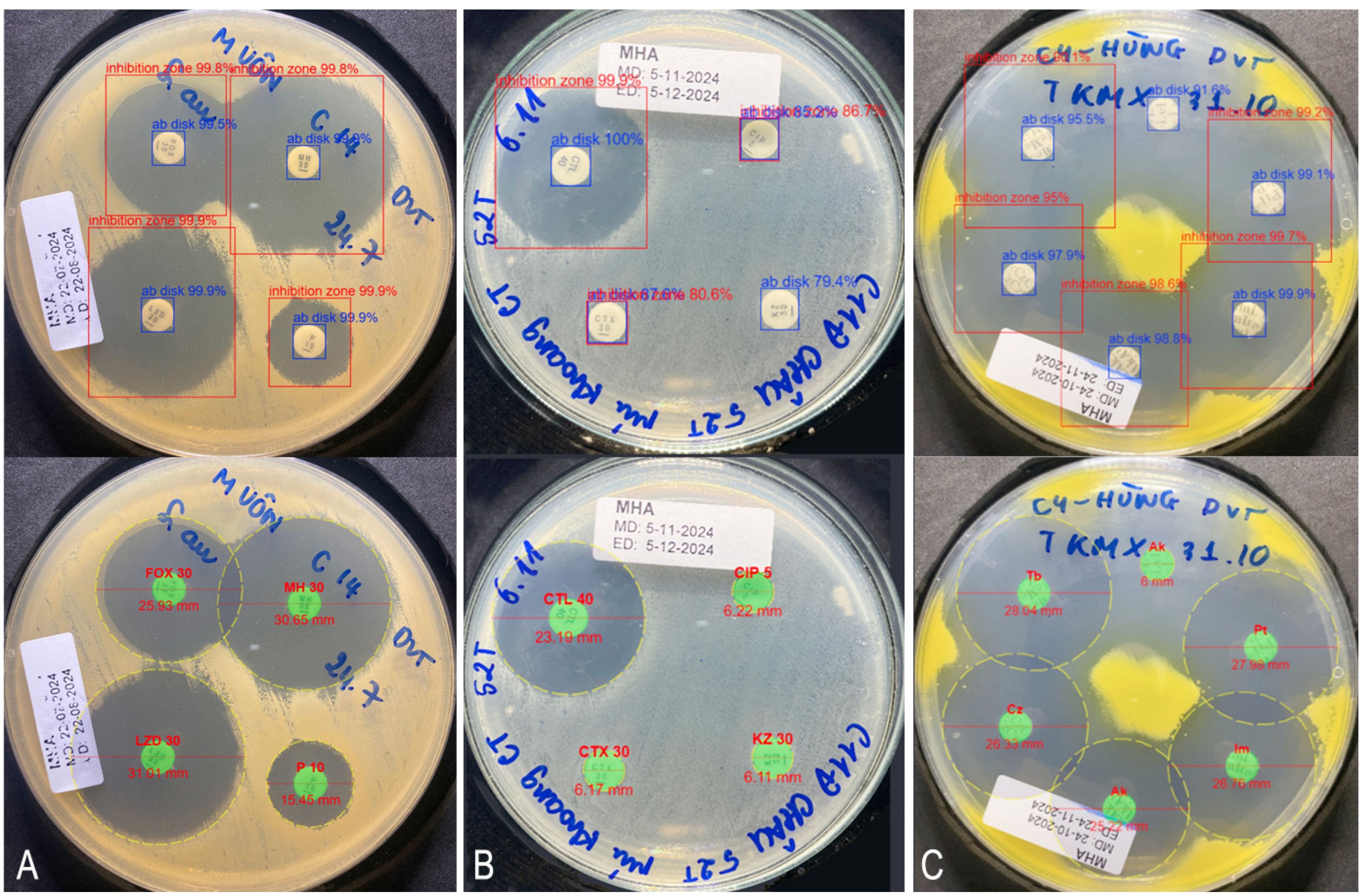
99.9% for object detection, with the highest scores inversely correlated with the number of antibiotic discs present on a single plate. Five percent of the images contained at least 1 object (ib_zone or ab_disc), with a confidence score below 80% (Table 1, Figure 3).

**Figure 3.** Confidence scores for antibiotic disc (ab_disc) and inhibition zone (ib_zone) detection across different Kirby Bauer test images.




**A:** High confidence score on Mueller-Hinton agar plate. **B:** High confidence score on blood agar plate. **C:** Low confidence score on Mueller-Hinton agar plate

**Figure 4.** Detection of the antibiotic discs, calculation of the inhibition zone diameter, and classification of abbreviations displayed on discs.




**A:** Detection and classification of all inhibition zones and antibiotic discs on the S. aureus AST plate. **B:** Missed detection due to absence of the inhibition zone on the E. coli AST plate. **C:** Missed detection due to the excessively large inhibition zones on P. aeruginosa AST plate. AST: antibiotic susceptibility test.

*Classification of the antibiotic abbreviation on disc and inhibition zone diameter calculator*

A total of 211 Kirby-Bauer test images were analyzed, comprising isolates of *S. aureus* (75, 35.54%), *E. coli* (61, 29.91%), *P. aeruginosa* (43, 20.38%), and *K. pneumoniae* (32, 15.17%). A total of 1,121 inhibition zones were examined from these images, and the AI-powered application successfully detected and identified 1,042 inhibition zones (92.95%). The AI-powered application did not detect 79 inhibition zones owing to two main issues: excessively large zones that merged and the absence of any inhibition zones (Figure 4). The AI-powered application and manual methods accurately detected all 1,121 antibiotic discs (100%).

The antibiotics used in this study were categorized into 16 drug classes based on their mechanisms of action, representing a comprehensive antimicrobial susceptibility testing (AST) profile for 42 antibiotics. The AI-powered application recognized that these antibiotics were associated with 60 of 63 unique commercial abbreviations displayed on the discs. Each antibiotic was represented by 1 or more commercial abbreviations, highlighting the variability in terminology. Notably, 14 antibiotics had 2 distinct commercial names, whereas 2 were associated with 3 different abbreviations. A detailed summary of the antibiotics, their classifications, and their abbreviations is provided in Table 2.

The diameter of the inhibition zone was calculated by determining the coordinates of the center of the bounding box around the antibiotic disc. The AI-powered application identified 1,121 center coordinates in the pixels. The pixel inhibition zone size was

**Table 2.** Summary of antibiotics, drug classes, and their commercial abbreviations classified by the AI-powered app.

| Drug class | Antibiotics | Commercial abbreviation | Number of abbreviations |
|---|---|---|---|
| **Aminoglycosides** | Amikacin | Ak, AK 30 | **2** |
| | Gentamicin | CN 10, GM 10 | **2** |
| | Gentamicin-high | CN 120 | 1 |
| | Tobramycin | Tb, TOB 10 | **2** |
| **Beta-lactam + inhibitors** | Ceftazidime/Avibactam | CZA 50 | 1 |
| | Amoxicillin/Clavulanic acid | AMC 30, AUG 30 | **2** |
| | Ampicillin/Sulbactam | SAM 20, AMS 20 | **2** |
| | Cefotaxime/Clavulanic acid | CTC 40, CTL 40 | **2** |
| | Piperacillin/Tazobactam | TZP 110, Pt | **2** |
| | Ticarcillin/Clavulanic acid | TTC 85 | 1 |
| **Cephems** | Cefazolin | KZ 30 | 1 |
| | Cefepime | Cm, FEP 30 | **2** |
| | Cefotaxime | CTX 30, Ct* | **2** |
| | Cefoxitin | FOX 30 | 1 |
| | Ceftaroline | CPT 30 | 1 |
| | Ceftazidime | CAZ 30, Cz* | **2** |
| | Ceftriaxone | CRO 30 | 1 |
| | Cefuroxime | CXM 30 | 1 |
| | Cefixime | CFM 5 | 1 |
| **Folate pathway inhibitors** | Trimethoprim/Sulfamethoxazole | SXT 25, Bt | **2** |
| | Trimethoprim | TM 5 | 1 |
| **Fosfomycins** | Fosfomycin | FOS 200 | 1 |
| **Glycopeptides** | Vancomycin | VA 30 | 1 |
| **Lincosamides** | Clindamycin | CD 2, DA 2 | **2** |
| **Macrolides** | Clarithromycin | CLR 15 | 1 |
| | Erythromycin | E 15 | 1 |
| **Monobactams** | Aztreonam | ATM 30 | 1 |
| **Nitrofurans** | Nitrofurantoin | F 300 | 1 |
| **Oxazolidinones** | Linezolid | LNZ 30, LZD 30 | **2** |
| **Penems** | Ertapenem | ETP 10 | 1 |
| | Imipenem | Im, IMI 10, IPM 10 | **3** |
| | Meropenem | MRP 10, MEM 10, Me* | **3** |
| **Penicillins** | Ampicillin | Am, AMP 10 | **2** |
| | Penicillin G | P 10 | 1 |
| **Phenicols** | Chloramphenicol | C 30 | 1 |
| **Quinolones** | Ciprofloxacin | CIP 5 | 1 |
| | Levofloxacin | LEV 5, Lv | **2** |
| | Moxifloxacin | MXF 5 | 1 |
| | Norfloxacin | NOR 10 | 1 |
| **Tetracyclines** | Doxycycline | DO 30, DXT 30 | **2** |
| | Minocycline | MH 30, MN 30 | **2** |
| | Tetracycline | TE 30 | 1 |

The table is sorted alphabetically by drug class and antibiotic names. Bold numbers indicate multiple commercial abbreviations. * Abbreviation label not recognized before HITL and fine-tuning process. AI: artificial intelligence; HITL: Human-in-the-loop.

**Table 3.** Comparison of inhibition zone diameters detected by AI and technicians, and their impact on interpretation.

| Difference in diameter | Inhibition zones (n = 1042) | Affecting interpretation | Effect type |
|---|---|---|---|
| ≤ 1 mm | 70 (6.72%) | 0 | – |
| > 1 mm but ≤ 2 mm | 29 (2.78%) | 2 | Sensitive to intermediate |
| Equal | 933 (89.54%) | 0 | – |
| > 2 mm but ≤ 3 mm | 6 (0.58%) | 2 | Resistant to intermediate |
| > 3 mm | 4 (0.38%) | 2 | Intermediate to sensitive |

AI: artificial intelligence.

calculated using these coordinates and the bounding-box inhibition zone. Most commercial antibiotic discs have a diameter of 6 mm.

The data of 1,042 inhibition zones identified by AI on the 4 bacterial AST results was used to calculate the diameters using the abovementioned formula that converts pixel-based measurements into millimeters. The application and technicians collected data simultaneously to assess the accuracy and deviation of the AI model (Table 3).

The results demonstrated that AI accurately matched the technician measurements in 933 cases (89.54%), indicating high precision. Minor differences of 1 mm or less were observed in 70 cases (6.72%); however, these discrepancies did not lead to changes in interpretation. Differences greater than 1 mm, but not exceeding 2 mm, were detected in 29 cases (2.78%), with 2 instances resulting in interpretation shifts from "sensitive" to "intermediate." Larger deviations, ranging between 2 mm and 3 mm, occurred in only 6 cases (0.58%) and affected 2 interpretations, causing a transition from "resistant" to "intermediate". The most significant discrepancies, exceeding 3 mm, were found in only 4 cases (0.38%); and all resulted in interpretation changes, shifting from "intermediate" to "sensitive".

These findings highlight the overall reliability of the AI model in detecting inhibition zone diameters. However, rare deviations exceeding 1 mm were shown to influence AST interpretations, underscoring the need for rigorous validation and periodic review to ensure AI's clinical applicability and decision-making accuracy.

### *Retraining using HITL and fine-tuning*

The technicians supervised the data analysis using the AI model to ensure accuracy. Inhibition zones, zone diameters, and antibiotic labels were reviewed to standardize the data and address cases of misidentification using the AI model. By using the initial dataset to analyze 211 evaluation images, 79 out of 1,121 inhibition zones, and 3 out of 65 antibiotic labels were incorrectly detected. A tool was developed to allow users to adjust and save supervised data into a database. The curated data were subsequently used to retrain and reevaluate the AI model. There were significant improvements upon reevaluation; undetected inhibition zones dropped to just 8 out of 1,121, and all 65 antibiotic labels were accurately identified and classified (Table 4).

### *The fully functional PWA "Disc Diffusion Reader"*

The PWA "Disc Diffusion Reader" was successfully developed and deployed at URL https://amr.ai.vn, providing a robust platform for analyzing ASTs. This "Disc Diffusion Reader" application can be installed and run on any device with an internet connection. PWA integrates advanced AI models to detect inhibition zones, measure their diameters, and accurately identify antibiotic discs. The application features a user-friendly interface that enables seamless image upload, real-time AI analysis, and manual data validation through the HITL approach.

Laboratory account management functions efficiently to manage user accounts and access controls using an email address as the username and a secure password. The laboratory data management system enables laboratories to streamline their organization and retrieve laboratory records. Image acquisition and management functions allow the seamless capture, storage, and processing of laboratory images. Technicians can easily capture multiple photos using the native camera app or upload images from other sources, such as USB cameras, ensuring secure storage in the laboratory directory with high privacy standards. Additionally, the application provides flexibility to edit,

**Table 4.** Evaluation of AI detection performance before and after the HITL process.

| Objects | Evaluation dataset | HITL process | |
|---|---|---|---|
| | | Before | After |
| Inhibition zones | 1,121 | 1,042 (92.95%) | 1,113 (99.28) |
| Antibiotic discs | 1,121 | 1,121 (100%) | 1,121 (100%) |
| Antibiotic abbreviation label | 65 | 63 (96.92%) | 65 (100%) |

AI: artificial intelligence; HITL: Human-in-the-loop.

update, or modify any information associated with the test images, enhancing the overall management efficiency (Figure 5).

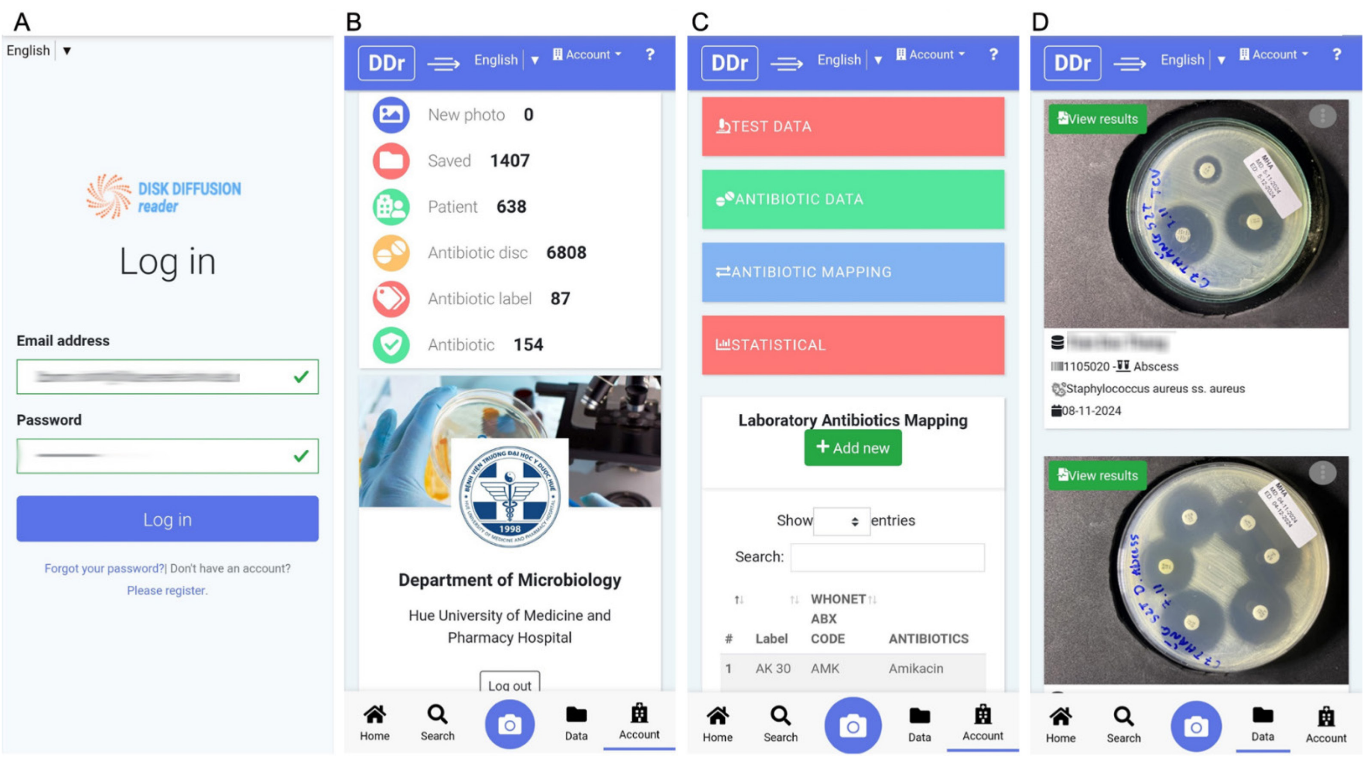
The system demonstrates robust performance with several key features that enhance the efficiency of the Kirby-Bauer test analysis. It automatically detects and measures inhibition zones, eliminates manual measurement errors, and classifies antibiotic discs while interpreting abbreviated labels with high accuracy. Integrating the CLSI/EUCAST breakpoint standards via the WHONET API enables automated and consistent breakpoint interpretation. A real-time alert system notifies technicians when the measured values approach critical thresholds, ensuring timely intervention. Additionally, the correction and standardization tool allows for the easy adjustment of misidentified data and database updates, ensuring the accuracy and adaptability of the system to evolving needs (Figure 6). The system demonstrated reliable performance in laboratory settings, capable of processing and analyzing up to 50 Kirby-Bauer test images per hour with minimal latency and high precision, particularly with HITL process involvement.

*Potential solution in real-world applications*

The "Disc Diffusion Reader" assessed 211 Kirby-Bauer test result images in real-world laboratory settings. Initially, AI analysis achieved an overall accuracy of 92.95% for detecting inhibition zones and 96.92% for identifying antibiotic abbreviation labels. After HITL corrections and iterative retraining, the performance of the AI model significantly improved, reaching a 99.28% detection rate for inhibition zones and 100% accuracy for antibiotic labels.

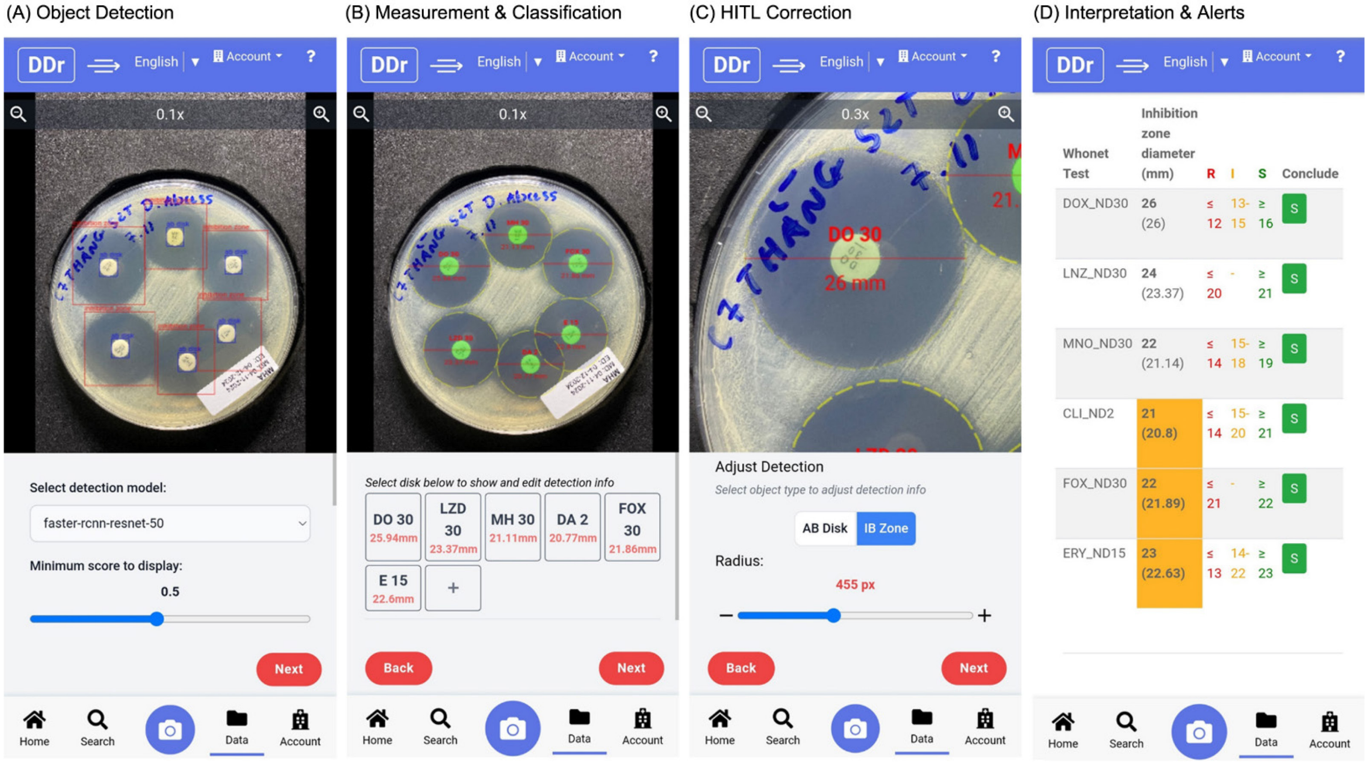
Real-world deployment provided further insights. The system effectively managed diverse datasets from various laboratories, showing adaptability to different test conditions and image qualities. The HITL approach reduced technician workload by 65% because manual corrections were required only for rare edge cases. Additionally, the tool sped up decision-making processes in clinical microbiology, reducing analysis time by 30%, compared to manual methods. These findings highlight the potential of the "Disc Diffusion Reader" to enhance AST workflows while maintaining high accuracy and consistency across different uses.

**Figure 5.** Laboratory account management features.




**A:** Authentication functionality. **B:** Laboratory dashboard overview. **C:** Laboratory data management system. **D:** Listing of laboratory test images.

**Figure 6.** Workflow of the AI-powered application for analyzing Kirby-Bauer test results.




**A:** Object detection. **B:** Measurement and classification. **C:** HITL correction. **D:** Interpretation and alerts. AB: antibiotic; DA: clindamycin; DOX: doxycycline; E: erythromycin; ERY: erythromycin; FOX: cefoxitin; IB: inhibition zone; I: Intermediate; LZD: linezolid; MH: minocycline; R: resistant; R-CNN: region-based convolutional neural network; RESNET: residual neural network; S: susceptible; WHONET: World Health Organization Network. AI: artificial intelligence; HITL: Human-in-the-loop. CLSI: Clinical and Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; GUI: dedicated graphical user interface; AST: antibiotic susceptibility testing.

## Discussion

The Kirby-Bauer or disc diffusion method is a traditional approach to AST that involves placing antibiotic discs on an agar plate inoculated with bacteria. It is valued for its simplicity, cost-effectiveness, and ability to provide precise visual results for antibiotic selection [18]. However, this method has limitations, including variability in results due to differences in the agar composition and incubation conditions. It is also less effective for testing slow-growing or fastidious organisms, is time-consuming, and heavily reliant on the accuracy and consistency of the technicians' interpretation. Automated systems, such as VITEK 2 (bioMérieux, Marcy-l'Étoile, France), BD Phoenix (Becton Dickinson, NJ, USA), and MicroScan Rapid ID (Beckman Coulter, CA, USA); offer faster results, often within hours, along with high accuracy, consistency, and integrated data management tools for tracking and interpreting results. These methods also reduce the workload of laboratory technicians by automating many steps in the testing process. Despite these advantages, automated systems are expensive to set up and maintain, may not detect rare or emerging resistance mechanisms, and require regular calibration and maintenance to ensure accuracy [19–21].

VITEK 2 is a fully automated commercial AST system that provides rapid results based on microbial growth kinetics rather than disc diffusion. It is widely adopted in clinical laboratories due to its high accuracy and standardized workflows. However, VITEK 2 involves significant costs related to acquisition, proprietary consumables, and maintenance; making it less accessible for laboratories in low-resource settings. Additionally, it relies on predefined panels of antibiotics, limiting flexibility when testing custom antibiotic panels or novel resistance patterns.

Although the disc diffusion method remains a cost-effective and accessible option, automated systems significantly improve speed, precision, and efficiency. However, their high cost and complexity may limit their suitability for laboratories in developing countries and low-resource settings where the Kirby-Bauer method continues to play a vital role.

Several automated or semi-automated software solutions utilizing "computer vision" have been developed to assist in reading and analyzing the Kirby-Bauer test results. These software tools can be integrated with hardware to support disk imaging using cameras, such as the Scan 4000 (Interscience, Puycapel, France), BIOMIC V3 (Giles Scientific, CA, USA), ADAGIO (BIO-RAD, Marne La Coquotte, France), and SIRscan 2000 (i2a, Montpellier, France). Unfortunately, as noted in previously published reviews, most integrated systems remain prohibitively expensive for many laboratories; a situation that has persisted over the years [22,23].

AntibiogramJ is an open-source software designed to semi-automatically analyze disk diffusion test images by detecting, measuring, and categorizing inhibition zones. This software, developed in Java, analyzes the images captured by camera-equipped devices, including smartphones. The fully automated process achieved 87% agreement with expert microbiologists and allowed easy manual correction for inaccurate readings to ensure accurate results [24]. AntibiogramJ is designed to analyze disc diffusion assay results from digital images. While it provides a cost-effective alternative to commercial systems, it necessitates manual image input and processing, which can introduce variability based on user expertise. Its reliance on standard image processing techniques may limit adaptability to variations in image quality, lighting conditions, and disc placement. In contrast, the "Disk Diffusion Reader" leverages AI for automated detection and analysis, reducing human intervention and enhancing consistency and accuracy. Moreover, the "Disk Diffusion Reader" integrates an HITL learning approach, enabling continuous improvement through real-world feedback—a feature not present in AntibiogramJ. Other open-source solutions, such as the mobile application developed by Pascucci *et al.*, represent promising alternatives that utilize machine learning and image processing to provide a more affordable and accessible approach. However, this solution may face comprehensive performance and robustness challenges across a diverse range of images and settings [25]. Table 5 summarizes the similarities and differences between applications using computer vision to analyze images of AST results using the Kirby-Bauer method. While AST systems like AntibiogramJ, VITEK 2 (bioMérieux, Marcy-l'Étoile, France), and BIOMIC V3 (Giles Scientific, CA, USA) have advanced the field of AST, they present limitations in cost, adaptability, and ease of use. The proposed "Disc Diffusion Reader: addresses these challenges by offering a cost-effective, adaptable, and user-friendly solution, leveraging AI technology to enhance accuracy and efficiency in diverse laboratory settings.

AI-enabled solutions like the "Disk Diffusion Reader" offer significant potential to enhance AMR surveillance, particularly in resource-limited settings. This application streamlines the analysis of AST results, reducing dependency on specialized expertise. However, ongoing advancements are crucial to maximize its impact. The key challenges include addressing image variability, enhancing the robustness

**Table 5.** Features available for Kirby-Bauer test image analysis in the software/application tool.

| **Criteria** | **AntibiogramJ Alonso *et al.* [24]** | **AST Image processing Pascucci *et al.* [9]** | **Disc Diffusion Reader** |
|---|---|---|---|
| Open-source | × | × | Free access |
| Operating system | Independent | Independent | Independent |
| Dependencies | Java | C ++ and Python | PHP and Python |
| AI-powered integrated | - | × | × |
| Works with images captured with any camera device | × | × | × |
| Image preprocessing | × | × | × |
| Determination of antimicrobial-disk codes | × | × | × |
| Identification algorithm | Manual | Automatic | Automatic |
| HITL and fine-tuning | - | - | × |
| Visualization of breakpoints | × | × | × |
| CLSI/EUCAST support | × | × | × |
| Breakpoint auto-updates | - | × | × |
| Devoted GUI | × | × | × |
| Database support | × | × | × |
| Image annotation | × | × | × |
| Reports | × | × | × |
| Import/export data | × | × | × |
| Data statistical analysis | - | - | × |
| Cross-device | - | - | × |
| Searching | × | × | × |
| Cost | Free | Free | Free |

AI: artificial intelligence; HITL: human-in-the-loop. CLSI: Clinical and Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; GUI: dedicated graphical user interface; AST: antibiotic susceptibility testing.

of AI models, and expanding datasets to improve both accuracy and reliability. By overcoming these limitations, AI-powered tools can become more accessible and effective, providing vital support for AMR monitoring in developing countries.

The region proposal network (RPN) identified potential regions containing antibiotic discs, which were then analyzed to predict their locations and measure inhibition zones. Training the RPN involves classifying regions, refining predictions, and improving reliability through data augmentation. ResNet-50, a deep neural network, was used to extract detailed features from the images, making it effective for understanding complex patterns. Another model used was RetinaNet, which helped detect hard-to-spot objects by balancing the class representation and enhancing the detection accuracy. MobileNetV2, a lightweight and efficient model, classified antibiotic labels. Its compact design and speed make it ideal for quick and accurate label recognition, even in settings with limited resources. These approaches collectively support the accurate analysis of Kirby-Bauer test results [14,26].

Our study shows that AI-powered applications have the potential for high performance in Kirby-Bauer test image analysis. However, the application faces challenges that can affect its accuracy and usability. A key issue is the misidentification of antibiotic discs owing to manufacturers' variations in design, size, color, and labeling. Unique abbreviations or formats not included in the training data may lead to errors, slowing workflows in unfamiliar laboratories. Expanding the training dataset to cover more manufacturers can improve generalization and reduce misidentification. Another challenge is ensuring precise measurement conversion for measuring the inhibition zones. Low-resolution images, uneven camera angles, and poor lighting can distort measurements and affect results. Standardizing imaging protocols such as consistent camera alignment, distance, and lighting can mitigate these errors. Pre-processing tools to correct distortions and advanced calibration techniques, such as using reference markers, can further enhance the accuracy. Improving the antibiotic database and leveraging the HITL feedback system will help the application adapt to diverse conditions, ensuring reliable performance across various laboratory settings.

The present study demonstrates that our AI-driven “Disc Diffusion Reader” effectively automates and standardizes the Kirby-Bauer method, reducing interpretive variability and technical demands. However, several limitations warrant consideration. First, our results are based on a single-center dataset, raising concerns about dataset bias. Laboratories from different geographic regions may use agar plates with variable compositions and different antibiotic disc manufacturers, or operate under distinct climate conditions, which can influence imaging and detection accuracy. Future multi-center collaborations encompassing diverse laboratory settings would help validate and refine the model, ensuring more robust generalizability [4,5].

Looking ahead, future work will focus on two main areas. The first involves extending the “Disc Diffusion Reader” to fungal AST. Many clinical laboratories face similar challenges when interpreting antifungal disc diffusion tests, and initial explorations suggest that our AI-driven platform could similarly reduce human error and improve throughput in mycology. Indeed, emerging AI and imaging applications for yeast and mold identification are already showing promise in preliminary studies [8,27]. Second, we aim to provide offline functionality for the “Disc Diffusion Reader” in low-resource settings with limited or inconsistent internet connectivity. Much of the computational load (e.g., model inference and HITL data synchronization) requires at least periodic online access [25]. By incorporating optimized on-device inference engines and intermittent data synchronization methods, we envision a more resilient, location-agnostic solution to support laboratories in remote areas.

## Conclusions

We have successfully developed a PWA app integrating an AI model that reads Kirby-Bauer AST results accurately, quickly, and efficiently. Although the VITEK 2, BD Phoenix, and Microscan systems are optimal, the Kirby-Bauer method continues to be used widely to monitor antibiotic resistance in developing countries. The application of AI to support the reading of results is highly valuable. The dataset, based on antibiotics available in our laboratory, may face recognition challenges in other laboratories. However, diverse antibiotic labels can be gathered to complete the dataset using the integrated HITL tool for broader recognition in the future.

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### Data availability
The datasets utilized and/or analyzed during this study are available from the corresponding author upon reasonable request.

### Authors' contributions
HBN, study design, data analysis, manuscript draft and refinement; TTU, TKLN, laboratory work such as antibiotic susceptibility testing and capturing photos; HBN, TLP, AI model integration, PWA development. All authors have read and accepted the manuscript.

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### Conflict of interests
No conflict of interests is declared.

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