

Using deep learning methods to automatically interpret blood culture Gram stains

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ABSTRACT

Background: Gram staining of the smear prepared as soon as the growth signal is received in automated blood culture systems is very important in terms of providing the first critical information for the clinician to plan the appropriate treatment. However, microscopic interpretation of Gram-stained smears is one of the most time-intensive processes. At this stage, the use of deep learning techniques will be beneficial for us.

Methods: In the blood cultures sent to İzmir Bakırçay University Çiğli Training and Research Hospital Microbiology Laboratory during the project period, two smears with the same thickness were prepared with the same technique from those with positive growth signals. The smears were stained on a fully automated Gram staining device and digitized with a slide scanning and imaging device. After manual labeling of the micro-organisms in the images obtained, work was carried out on the training set using image processing and current deep learning techniques, and the analysis results were supported by the test set.

Results: We used the deep learning models xresnet50, resnet50, xresnext50, and mobilenetV3. The results indicate that it may be possible to develop a blood culture slide evaluation system using a deep learning model, particularly outside laboratory working hours.

Conclusions: Developing an automated system for Gram staining interpretation is crucial for ensuring uninterrupted laboratory operations both during and outside working hours. This will also contribute to antimicrobial stewardship by reducing the time it takes for a laboratory to issue its first report after a positive blood culture signal.

Keywords: artificial intelligence, automated microscopy, blood culture, deep learning, Gram stain

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INTRODUCTION

Bloodstream infections (BSI) are rapidly progressive infections with mortality rates of approximately 30-40%, and delayed initiation of antimicrobial therapy leads to an increase in mortality of up to 10% per day [1-4]. After a positive growth signal in blood cultures, the growth period for micro-organisms on culture plates can take 24-48 hours. Therefore, Gram staining of the smear prepared as soon as a growth signal is detected in automated blood culture systems is crucial as it provides the clinician with the first critical information to plan appropriate treatment [5]. However, microscopic interpretation of Gram-stained smears is one of the most time-intensive and time-dependent processes in the clinical microbiology laboratory. Consolidation of hospital systems, increasing workloads, and the lack of a sufficient number of Medical Microbiology specialists in the field

to carry out this work 24/7 create a need for support in the evaluation of Gram stains to strengthen and complement this manual test [6]. At this stage, the use of deep learning techniques may benefit us.

Deep learning is a subdivision of machine learning that is inspired by the human nervous system and deals with the functioning of the brain. The foundation of artificial neural networks, which imitate the human nervous system, was laid in the 1940s, and in the 1980s, studies such as redistributed processing and back propagation algorithms formed the basis of today's deep learning studies. In the 2012 Large Scale Visual Recognition Competition (ImageNet-2012), the deep learning basic architecture convolutional neural network won the first prize, which further increased the popularity of deep learning [7]. These studies and achievements have led to the intensive use of deep learning techniques in many fields

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such as autonomous vehicles, space and defence industry and medicine. Deep learning techniques are used in much research in the field of medicine (radiology, pathology, microbiology, etc.) to support clinical diagnosis and treatments [8]. However, the number of scientific studies on the automatic interpretation of blood culture Gram stains with deep learning is very limited worldwide and there is no study conducted in our country.

Gram stained preparations are generally examined with the use of immersion oil in 100x lenses. The preparation prepared from clinical specimens can only be adequately visualised in a very narrow focal area. In addition, Gram-stained preparations show highly variable background staining in all areas. This staining often mimics the shape and colour of bacterial cells. Automated interpretation of Gram staining requires automated slide imaging and automated image analysis. The applications of automated slide scanners in clinical microbiology remain limited due to the technical difficulties [9].

By making the main subject of our study feasible, it will be possible to detect the early/pre-causative micro-organism in BSIs in healthcare institutions. This is particularly important in institutions where there are not enough medical microbiology specialists available to evaluate Gram-stained preparations outside of laboratories working hours (5 p.m. to 8 a.m.). Rapid reporting of Gram staining results will enable early treatment. The time of initiation of treatment is the most important component in the effective treatment of infection. Early treatment is a measure that significantly reduces mortality and morbidity, especially in serious infections such as BSI. In this study, we planned to develop an automatic Gram stain analysis based on deep learning.

METHODS

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of İzmir Bakırçay University Scientific Research Projects Coordination Unit (Date 28.09.2022/ No-714).

Slide collection and slide classification

In our laboratory, internal quality control of Gram staining is performed daily using ATCC 25922 *E. coli* (for Gram-negative staining) and ATCC 29213 *S. aureus* (for Gram-positive staining).

At least 2 smears of the blood cultures sent to İzmir Bakırçay University Çiğli Training and Research Hospital Medical Microbiology Laboratory during the project period were prepared with the same technique and with the same thickness. The smears were prepared within 10 minutes after the positive signal and stained using a fully automated Gram Staining device [PREVI® Color

Gram (BioMérieux, Marcy L'Etoile, France)]. All slides were evaluated by two different medical microbiology specialists as part of the routine work algorithm, and the results were quickly reported to the relevant clinics. After Gram staining, the slides were covered with coverslips and stored until scanned with a slide scanner.

The prepared Gram-stained blood culture slides were scanned using an automatic slide imaging platform Panoramic® 250 Flash III- (3dhitech, Hungary) digital scanner with 40x magnification and digitised with Slideviewer version 2.5.0 (3dhitech, Hungary) imaging software. Then, Gram (-) bacilli, clustered Gram (+) cocci, chained Gram (+) cocci, Gram (-) coccobacilli and yeast-like fungi were used in the training set in order to recognise morphologies representing micro-organisms that may be the causative agents of BSI [10]. After manual labelling (segmentation and classification) of the micro-organisms in the acquired images, the labelled images were divided into training and test sets. Image processing and current deep learning techniques were applied on the training set and the analysis results were supported with the test set. Our trained model was then evaluated in terms of accuracy compared to manual classification.

Data collection process

Blood culture bottles [(BacT/ALERT FA/aerobic or BacT/ALERT FA/anaerobic) (BioMérieux, Marcy L'Etoile, France)] sent from the clinical services of İzmir Bakırçay University Çiğli Training and Research Hospital are incubated in the automated blood culture system [BacT/ALERT® Blood Culture System (BioMérieux, Marcy L'Etoile, France)] in the Clinical Microbiology Laboratory. In the routine process, the bottles with a positive growth signal are inoculated on appropriate solid media and slides are prepared for Gram staining. Blood culture slides with no growth detected were also prepared to be used as negative controls. Between June 2022 and May 2023, a total of 170 Gram-stained slides were prepared from blood culture bottles.

Slides were prepared during routine laboratory work and no pre-selection was made before collection. The most common results we encountered in blood cultures sent to our laboratory were those that did not show a growth signal and were considered negative. Therefore, 22 slides with no growth signal were included in the study as negative controls.

Our data collection process consisted of a total of 164 slides, which were divided into two groups: 94 slides for the training set and 70 slides for the test set. To ensure a comprehensive representation of each slide, a region was selected from each to cover the entire slide. These selected regions were then extracted into 3000 x 3000-pixel patches using the Python Open Slide library. All patches were then resized to 300 x 300 pixels, result-

ing in the final dataset. The complete dataset consists of 137,900 images distributed as 92,200 images assigned to the training set and 45,700 images to the test set. Each image is labelled with one of the classes listed in Table 1, which provides a comprehensive distribution of images for each class.

Deep learning models

In our study, we present the training process for four different Deep Learning models using a dataset of 300 x 300-pixel images. Three of these architectures are based on the award-winning ResNet architecture, which won first place in the 2015 ImageNet competition. The fourth architecture we use is MobileNet, known for its efficiency and suitability for deployment on mobile devices. ResNet50 is a powerful Deep Learning model [11], which stands for '50-layer Residual Network'. XResNet50 is a variant of the original ResNet50 that incorporates the idea of 'Extreme Residual Learning', with improved accuracy [12, 13]. It has become a successful choice for image classification on smaller images. XResNext50 is another variant of ResNet50 that adopts the concept of ResNext architecture. ResNext models utilize cardinality, which can be considered as a form of ensemble learning [13, 14]. We included xResNext50 in our study to investigate the potential advantages of using our dataset with relatively lower resolution images. MobileNetV3 provides efficient neural architectures optimized for mobile and embedded devices. In addition to its lightweight design and competitive accuracy, it enables real-time inference [13, 15].

Data preprocessing and training process

Data preprocessing was performed by resizing all images to the target resolution of 300 x 300 pixels. Training was performed using the Fastai_v2 library [16] on a device equipped with an Nvidia GeForce RTX 3080 8 GB graphics card, Intel Core i7 11800H processor and 32 GB RAM. The images used for training and validation of the models were not subjected to any preprocessing technique and no data augmentation technique was used. The model with the best performance was achieved with a tuned training process using the Fastai library.

Evaluation Process

The performance of classification models can be interpreted by using the 'confusion matrix' used to measure image classification performance. Using the values in the matrix, parameters such as accuracy, precision, sensitivity, recall, F1-score can be calculated.

Accuracy measures the proportion of correctly classified samples among all samples. Precision measures the accuracy of positive predictions, calculating the ratio of true positives to the sum of true positives and false posi-

tives. Recall, also known as Sensitivity or true positive rate, measures the ability of the classifier to recognize all positive samples and is calculated as the ratio of true positives to the sum of true positives and false negatives. The F1 Score provides a harmonic means of precision and sensitivity, providing a balanced assessment and is particularly useful when working with unbalanced data sets. In addition, the Receiver Operating Characteristic (ROC) is a probability curve, with the Area Under the Curve (AUC) representing the degree or measure of separability. The metric called Receiver Operating Characteristic Area Under the Curve (ROC AUC) is used in binary classification tasks, applicable to certain types of image classification scenarios. This metric evaluates the ability to distinguish between positive and negative samples and calculates the true positive rate by plotting it against the false positive rate at different classification thresholds. A higher ROC AUC value indicates a better discrimination ability of the classifier.

RESULTS

Slide collection and slide classification

Of the 170 Gram-stained slides prepared from blood culture bottles during the study period, six slides were excluded from the study due to the inability to obtain images related to staining. Therefore, a total of 164 slides evaluated by two different medical microbiology specialists were used in the study, of which 94 slides were used as a training set and 70 slides as a test set. While the number of yeast-like fungi slides was 52, 30 slides were Gram (+) cocci in chains, 22 slides were Gram (+) cocci and Gram (-) bacilli in clusters, and 16 slides were Gram (-) coccobacilli (Table 1).

Data collection process

In the dataset consisting of 137,900 images, 27,400 images were negative, while the number of Gram (-) bacilli images was 26,300, the number of clustered Gram (+) cocci images was 23,600, the number of yeast-like fungi images was 20,700, the number of Gram (-) coccobacilli images was 20,400 and the number of chained Gram (+) cocci images was 19,500 (Table 1).

Deep learning models

When the training times of the deep learning models were evaluated individually, mobilenetV3 completed the training in the shortest time with 4 hours, and this time was measured as 26 hours with resnet50, which was found to have the longest training time. With xresnet50 and xresnext50, the training time was 19 and 18 hours, respectively.

Table 1. Numerical distribution of images and slides for each label in the dataset

Label	Training set		Testing set		Total	
	Images	Slides	Images	Slides	Images	Slides
Negative	19,300	16	8,100	6	27,400	22
Yeast-like fungi	14,000	22	6,700	30	20,700	52
Gram (-) bacilli	17,100	14	9,200	8	26,300	22
Gram (-) cocco-bacilli	13,500	10	6,900	6	20,400	16
Chained Gram (+) cocci	13,000	16	6,500	14	19,500	30
Clustered Gram (+) cocci	15,300	16	8,300	6	23,600	22
Total	92,200	94	45,700	70	137,900	164

Data pre-processing and training process

The fact that MobileNetV3 completes the training process in just 4 hours can be seen as a notable advantage. Thanks to this advantage, MobileNetV3, as a lightweight model, has been identified as an excellent choice for scenarios where computing resources are limited or real-time performance is very important.

Evaluation process

After the training process was completed, we obtained a confusion matrix for each model (Figure 1) and comprehensive tables showing the results of all evaluation metrics (Table 2). With the data in these confusion matrices, all key performance metrics were calculated. After analysing the performance metrics presented in Table 2, it was observed that each model (resnet50, xresnet50, xresnext50, and mobilenetV3) exhibited strengths and weaknesses in various classification tasks.

According to the Accuracy values, the xresnet50 model was evaluated as the best performing model with a score of 0.99. With this model, a remarkable performance was observed in terms of correctly classifying more than one class. The other models also showed remarkable accuracies (0.98, 0.98, and 0.93, respectively), but they lagged behind xresnet50 (Table 2).

When Precision, which is calculated as the ratio of true positives to the sum of all positives, is analysed, the xresnet50 and xresnext50 models consistently achieved scores of 0.99 and above in all classifications. This shows their ability to make fewer false positive predictions and provide accurate classification. The precision results are shown in Table 2. When Sensitivity and Recall were evaluated, the xresnet50 and xresnext50 models maintained Recall values of 0.99 and above for most classification labels when recognising true positive examples. This indicates their ability to make fewer false positive predictions and provide accurate classification (Table 2). The F1-score provides a balanced measure of a model's Precision and Recall values. In this context, xresnet50 showed balanced and strong performance, performing exceptionally well on all labels. The MobilenetV3 model showed a performance gap especially for the chained Gram (+) cocci and clustered Gram (+) cocci labels (Ta-

ble 2). When the ROC-AUC scores are analysed, the xresnet50 and xresnext50 models are seen as models with excellent discrimination capabilities. These models appear to be effective in discriminating between positive and negative examples, consistently achieving high scores across all classification labels. The resnet50 and mobilenetV3 models also performed well in this respect, but slightly behind the leading models (Table 2).

DISCUSSION

Artificial Intelligence (AI) is a branch of science that aims to equip computer systems with human-like thinking and problem-solving capabilities, and aims to develop algorithms and models that can perform data analysis, learning, decision-making processes and even problem solving similar to human intelligence. Machine Learning (ML), a subset of artificial intelligence, involves enabling computer systems to automatically learn from data and improve their experience. It covers various algorithms such as linear regression, decision trees, support vector machines that can detect patterns, make predictions, classify data and make decisions based on data analysis. Deep Learning, a subfield of ML, uses multi-layered artificial neural networks to automate the process of learning from data. By processing data through these deep neural networks, complex patterns and features can be identified. Deep Learning has achieved impressive success in tasks such as image and speech recognition, natural language processing, game playing, and autonomous driving [17-19].

The main interest of artificial intelligence in the field of medicine is to develop methods that can provide diagnosis and treatment recommendations. The use of artificial intelligence studies, especially deep learning techniques, has started to increase rapidly after 2012. Especially in recent years, artificial intelligence technologies have been expanding into areas that are traditionally considered to be the expertise of humans only, with the latest developments in digitised data collection, ML and computing infrastructure [20].

Artificial intelligence applications can be utilised for each step in the diagnostic process in clinical microbiology, which is divided into preanalytical, analytical and

Table 2. Precision, Sensitivity, F1-score and ROC-AUC performance of each model

	Precision				Sensitivity			
	resnet50	xresnet50	xresnext50	mobilenetV3	resnet50	xresnet50	xresnext50	mobilenetV3
Negative	0.98	0.99	0.98	0.97	1.00	1.00	1.00	1.00
Yeast-like fungi	1.00	0.99	1.00	1.00	0.99	0.99	0.90	0.92
Gram (-) bacilli	0.99	0.99	0.99	0.96	0.95	0.99	0.97	0.92
Gram (-) cocco-bacilli	0.99	0.99	0.93	0.95	1.00	0.99	1.00	1.00
Chained Gram (+) cocci	0.98	0.99	0.99	0.77	0.99	0.99	0.98	0.93
Clustered Gram (+) cocci	0.96	0.99	0.96	0.95	0.99	0.99	0.99	0.83

	F1-score				ROC-AUC			
	resnet50	xresnet50	xresnext50	mobilenetV3	resnet50	xresnet50	xresnext50	mobilenetV3
Negative	0.99	1.00	0.99	0.98	0.9978	0.9992	0.9979	0.9942
Yeast-like fungi	0.99	0.99	0.95	0.96	0.9944	0.9955	0.9788	0.9759
Gram (-) bacilli	0.97	0.99	0.98	0.94	0.9733	0.9933	0.9837	0.9560
Gram (-) cocco-bacilli	1.00	0.99	0.97	0.97	0.9989	0.9964	0.9937	0.9942
Chained Gram (+) cocci	0.99	0.99	0.98	0.84	0.9937	0.9953	0.9891	0.9412
Clustered Gram (+) cocci	0.97	0.99	0.98	0.89	0.9886	0.9951	0.9928	0.9118

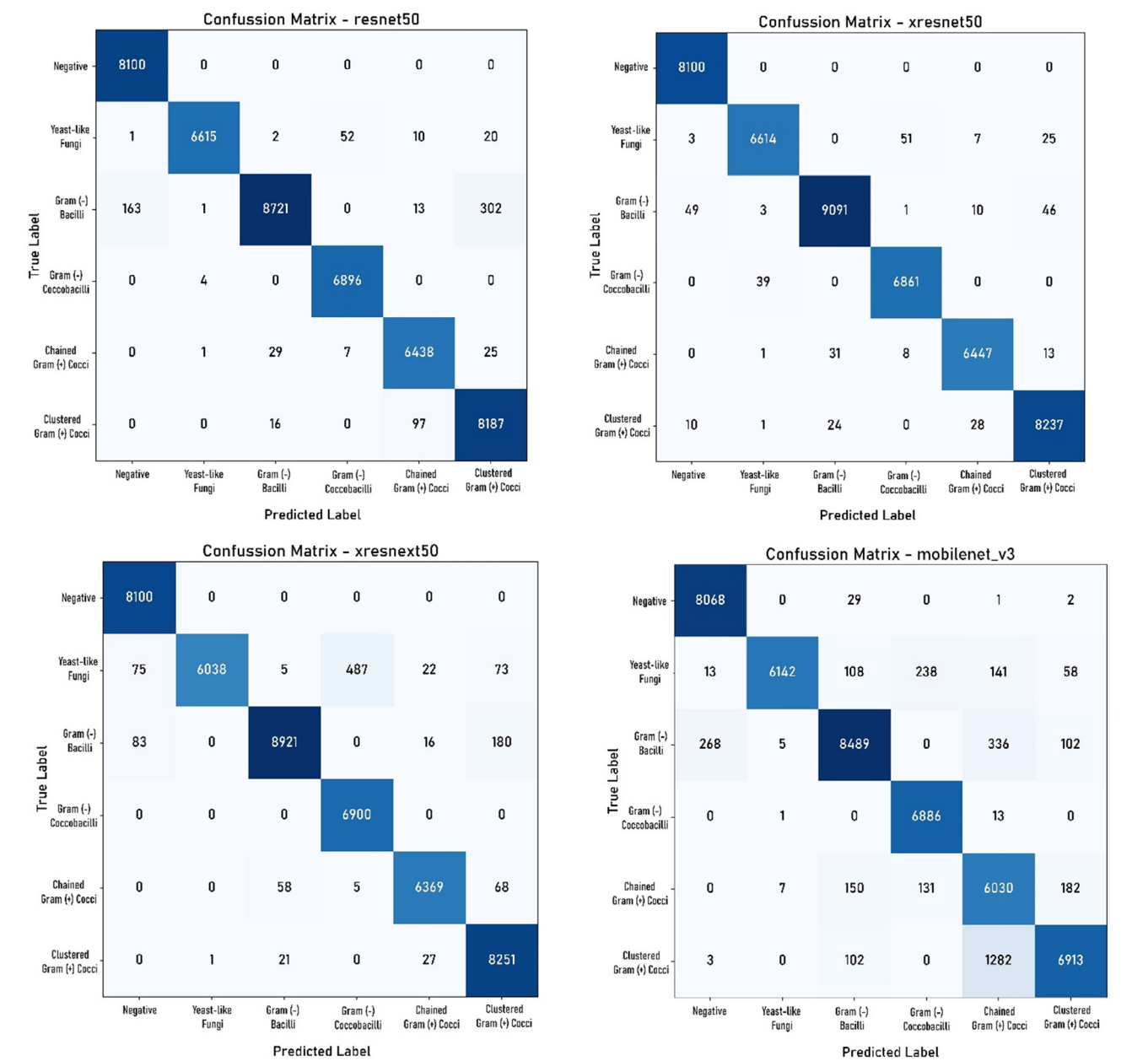


Figure 1. Confusion matrices for each deep learning model used to analyze the digitized Gram-stained smears

postanalytical steps [21]. Especially in studies evaluating the analytical process, digital imaging techniques and deep learning have been studied. In the studies, high-resolution images were obtained from slides with automated microscopy and Gram staining could be categorised with high sensitivity and specificity [22-25].

Interpretation of the image forms the basis of clinical microbiological diagnostic methods. For example, Gram stains, stool and blood smears should all be interpreted by a Medical Microbiology specialist. These specimens provide critical diagnostic information such as the presence and classification of micro-organisms, the inflammatory response of the host and the determination of sample quality. Taken in a clinical context, this information helps to determine whether an infection is present, often guiding treatment [22]. Automatic interpretation of blood culture Gram stains [23], diagnosis of bacterial vaginosis from smear samples classified according to Nugent scores [26], detection of *Plasmodium* species in thick and thin blood smears [27], detection of *Mycobacterium tuberculosis* on slides prepared from sputum samples [28] are examples of applications.

Bloodstream infections (BSI) are rapidly progressing infections with high mortality rates and require rapid initiation of antimicrobial treatment. After a positive growth signal in blood cultures, the result of Gram staining of the smear is of critical importance in planning the appropriate treatment, since the time of growth in culture plates may take 24-48 hours. Microscopic interpretation of Gram-stained smears continuously and without interruption is one of the most time-intensive and time-dependent processes in the clinical microbiology laboratory and is often not possible due to the insufficient number of Medical Microbiology specialists. Especially outside laboratories' working hours (between 5 p.m. and 8 a.m.), blood culture smears that cannot be evaluated by a medical microbiologist are reported to the clinician after working hours begin. This situation can be associated with increased mortality due to delays in treatment initiation. At this stage, there are studies that have benefited from the use of deep learning techniques. In these studies, sensitivity and specificity values for blood culture smears were found to be around 90% [23, 29].

In a study by Smith et al. [23], automatic interpretation of blood culture Gram stains was aimed by using Inception 3.0, a deep learning model which is superior in visual categorisation. According to the results of the study, approximately 95% classification accuracy was found in all Gram stain categories. The deep learning models xresnet50, resnet50 and xresnext50, which we used in our study, achieved >98% accuracy. Only the accuracy rate obtained with mobilenetV3, which is the 4th model we used in the study, remained at 93%.

In our study, the performance criteria of all deep learning models were evaluated (Table 2). The highest "Accuracy" value was calculated as 0.99 with xresnet50. The lowest accuracy rates were obtained with mobilenetV3, which had a successful prediction rate of 0.77 for Gram-positive cocci, and xresnext50, which had a successful prediction rate of 0.93 for Gram-negative cocci. Sensitivity rates, defined as the ability to identify all positive examples, were low for the MobileNetV3 model in the yeast-like fungus, Gram (-) bacillus, chained Gram (+) cocci, and clustered Gram (+) cocci labels. Among all these different performance values, the xresnet50 model demonstrated exceptional performance across all labels, achieving a high F1-score. In particular, the mobilenetV3 model showed a performance gap with a lower F1-score for chained Gram (+) cocci and clustered Gram (+) cocci labels.

Fisher et al. [29] evaluated the performance of automatic digital imaging of Gram-stained slides against manual microscopy and found a sensitivity rate of 99.3% for blood culture slides. Xresnet50 and xresnext50, the deep learning models we used in our study, showed sensitivity values of 99% and above for most classification labels. This shows their ability to make less false positive predictions and provide accurate classification.

Panicker et al. [28] studied a deep learning based method for the detection of tuberculosis bacilli in stained images of sputum slides and found 97.13% sensitivity, 86.76% F1-score, and 78.4% accuracy. In a study in which AlexNet, VGG-16, Xception, ResNet-50, and DenseNet-121 deep learning models were used in the microscopic diagnosis of malaria, ResNet-50, a model we also used in our study, performed better than other models with an accuracy rate of 95.7% [30]. In a study by Jameela et al. [31] in which VGG-19, ResNet-50, ResNet-34, and VGG-16 deep learning models were used in the classification of malaria positive and negative blood smears, it was concluded that VGG19 performed significantly better than ResNet50, ResNet34, and VGG16 in the classification of blood smears with 0.97 accuracy, 0.98 sensitivity, and 0.94 F1-score. Again, three models such as VGG19, ResNet50, and MobileNetV2 were used in the study on automatic detection of malaria parasite in blood from Giemsa stained smears. The researchers reported that the performance criteria such as sensitivity, accuracy, and F1-score of the models were found to be 100% compared to the basic techniques [32].

The study has some limitations. One limitation of our study was that we only included morphologies representing micro-organisms that could be the most common BSI agent. We excluded clinically important but less common bacterial morphologies (such as Gram (+) bacilli) and slides suspected of polymicrobial infection from

our study. In future studies, we aim to eliminate these limitations by easily teaching our deep learning models less common morphologies and slides associated with polymicrobial infections. Additionally, we aim to further improve classification by increasing the number of slides in the training set. Another limitation is that we cannot predict how inaccurate predictions will affect clinical decisions. In order to obtain these data, studies in which test sets are expanded are needed.

In blood cultures sent to our laboratory, the most common results we encountered were those that did not show any growth signals and were considered negative. Following this, in order of frequency, were growths of Gram (-) bacilli, clustered Gram (+) cocci, and yeast-like fungi. These growth results were successfully identified by all models to identify the most common morphologies representing micro-organisms that may be causing BSI. The lowest accuracy rates were obtained with the MobileNetV3 model for Gram-positive cocci in chains and with the Xresnet50 model for Gram-negative coccobacilli. The Xresnet50 model demonstrated exceptional performance for all morphologies, achieving a high F1-score. The MobileNetV3 model showed a performance gap with a lower F1 score, particularly for chained Gram-positive cocci and clustered Gram-positive cocci labels. The findings of this study provide practical insights for selecting the more appropriate model based on specific use cases and requirements.

We can say that Gram staining and slide preparation have a significant effect on automatic slide scanning and imaging. In our study, we aimed to ensure that the slides did not show high variability in terms of smear area, thickness, position, and staining intensity. Therefore, it is very important that the slides are prepared using the same technique and by the same person, and that an automated Gram staining device is used for staining. We believe that this approach will increase the repeatability and accuracy of staining characteristics and provide standardization parameters.

CONCLUSIONS

In general, the study provides data on the performance of different machine learning models (ResNet50, XResNet50, XResNext50, and MobileNetV3) in various classification tasks. A comprehensive evaluation of metrics such as accuracy, precision, recall, F1-score, and ROC-AUC has revealed how these models perform in different scenarios. According to our results, xresnet50 and xresnext50 achieved higher accuracy, precision, recall, and F1-score, yielding more successful results compared to other models.

However, we can say that the field of machine learning is constantly evolving and that new models and techniques may emerge in the future that could surpass

the performance of the models evaluated in this study. Therefore, it is very important to continuously research and evaluate models with the latest technology.

Based on the results of our study, it may be possible to develop a blood culture slide evaluation system using a deep learning model, particularly outside laboratory working hours. Developing such a system is crucial for ensuring uninterrupted laboratory operations both during and outside working hours. This will also contribute to antimicrobial stewardship by reducing the time it takes for a laboratory to issue its first report after a positive blood culture. In this case, improving the performance of models such as MobileNetV3, which can be used on mobile devices and provide real-time results, would be a particularly appropriate strategy. To this end, it will be necessary to increase the amount of data by extending the training periods.

The comprehensive evaluation of ML models in this study provides valuable guidance and lays the foundation for further research and improvement in this field.

ABBREVIATIONS

AI – artificial intelligence
AUC – area under the curve
ML – machine learning
MR – multiple randomization
ROC – receiver operating characteristic
TQ – test of quality

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AUTHORS' CONTRIBUTION

RY: conceptualization, project administration, investigation, methodology, writing – original draft, writing – review & editing
KK: investigation, writing – original draft, formal analysis, methodology, writing – review & editing
MB: supervision, validation, writing – review & editing

CONFLICT OF INTEREST

None to declare.

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