

EARLY CLASSIFICATION OF GRAM-NEGATIVE
BACTERIA WITH COLONY IMAGING AND DEEP
LEARNING WITHOUT CODING EXPERIENCE

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Received on January 3, 2024
Presented by I. Ivanov, Member of BAS, on February 27, 2024

Abstract

Bacterial colony morphology is the first step in classifying bacterial species during the microbial identification process. It is very important to assess the morphology of bacterial colonies in a preliminary screening process to largely reduce the scope of possible bacteria species and increase work productivity in clinical bacteriology by making later identification more specific. However, making a decision about this topic requires sufficient clinical laboratory expertise. Teachable Machine[®] is a rapid, easy-to-use, web-based tool accessible to everyone that is used to create machine learning models. In this study, the performance of Teachable Machine[®] was assessed for cheap, rapid and practical identification of enteric and non-fermenting bacteria frequently isolated in microbiology laboratories. A total of 1202 colony images were used to train and validate the network's diagnostic performance. Additionally, 80 representative test images were used to assess performance. Level 1 was defined as *E. coli*-*K. pneumoniae*, Level 2 was defined as *P. aeruginosa*-*A. baumannii*, Level 3 was defined as enteric bacteria-non-fermenting bacteria and Level 4 was defined as differentiating these four pathogens from each other. Mean accuracy of Teachable Machine[®] for the defined classes was 96.7%, 94.1%, 94.3%, and 90.3% for Levels 1, 2, 3, and 4, respectively. General accuracy for classification of the 80 representative colonies was 82.5% and the hit rates were 85.0%, 100%, 75.0%, and 70.0% for *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *A. baumannii*, respectively. This cost-effective bacterial identification system, supported by deep learning, will be an important pioneer for a variety of applications

<https://doi.org/10.7546/CRABS.2024.04.04>

in clinical microbiology by reducing the identification process by a significant degree and automating identification of colonies without requiring a specialist.

Key words: bacteria classification, colony phenotype, machine learning

Introduction. For diagnosis and effective treatment of infectious diseases, it is necessary to rapidly and reliably identify the causative bacterial species. Identification of bacteria is a procedure that is expensive, difficult to practice and requires interpretation by expert individuals trained for long durations about the topic. If this stage can be performed with simpler and cheaper methods, it will become more applicable without requiring specialist individuals and expensive devices or infrastructure.

Machine learning may be used for analysis, classification and prediction of microbiological data. Currently, it is used in several areas like identification of microbiological pathogens, prediction of antibiotic resistance, microbiological data mining, optimization of microbiological production processes and bacterial genome analysis [1–3]. For analysis of information rich in terms of data, artificial intelligence (AI) is superior and this field will significantly affect clinical microbiology laboratories. Visual information like bacterial colony images are among this type of rich data cluster [4]. AI may shorten the identification process and increase accuracy rates of diagnosis by analyzing the similarities and differences between bacterial species. Image processing and AI approaches may assist in accelerating medical treatment by analyzing these images [5]. Without AI, this type of data is analyzed manually, with intense effort and significant education and experience is required to perform these tasks well.

Teachable Machine[®] is a software allowing the possibility to create, train and distribute special machine learning models. It has a simple drag and drop interface facilitating creation and training of models even by those with limited technical experience, allowing the creation of special models without writing code. This makes it possible for people with very little or no programming experience to create and use special machine learning models [6]. High-quality images may be taken of plates using commercial apparatus; however, these are expensive and their use is not practical [5, 7, 8]. Although AI support of easily accessible devices (computer, mobile phone cameras, simple light diffusers) may not increase diagnostic efficiency, it may be useful to improve diagnostic accuracy in situations where technical expertise is limited.

AI may be a method that can be used to identify bacteria. AI algorithms may be able to analyze features like morphology, dimensions, shape and colours of bacterial colonies by performing image analysis. These features play an important role in identification of bacterial species. The aim of this study is to evaluate the ability of the Teachable Machine[®] platform, which is a simple, fast and free artificial intelligence tool, to identify gram-negative bacteria from colony photographs. In this study, colonies formed by gram-negative bacteria were photographed and

this data was used to train Teachable Machine[®] software. Then the identification performance of the system was tested.

Material and methods. Identification and photography of bacteria.

The study included strains 100% identified as *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *A. baumannii*, identified with a BD Phoenix (Becton Dickinson, USA) automated bacteria identification device and routinely obtained from a variety of clinical samples between August 2022 and August 2023 in a clinical microbiology laboratory. In this order, 453, 360, 237 and 232 bacterial strains were inoculated using sterile loops on eosin methylene blue (EMB) (Salubris, Istanbul, Turkey) agar plates. The plates were incubated for 24 h in an aerobic environment at 37°C. When colonies began to display morphological characteristics, photographs were taken of the plates with an iPhone 13 or iPhone 14 Pro Max mobile phone using a light diffuser constructed as described by SMITH et al. [9]. With the aim of obtaining standard images, all photographs were taken in square format using $\times 2$ optical zoom. Finally, 1282 standard plate photographs with 3024×3024 resolution were obtained in JPEG format and a database was created.

Classification. Bacterial colony images were divided into four levels according to routine bacterial classification. Discrimination power between the classes created within each level was tested. Level 1 included two classes of *E. coli* and *K. pneumoniae*, Level 2 included two classes of *P. aeruginosa* and *A. baumannii*, Level 3 included two classes of enteric bacteria (*E. coli*, *K. pneumoniae*) and non-fermenting bacteria (*P. aeruginosa*, *A. baumannii*) and Level 4 included four classes of *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *A. baumannii* (Fig. 1). Additionally, with the aim of assessing the performance of the designed network

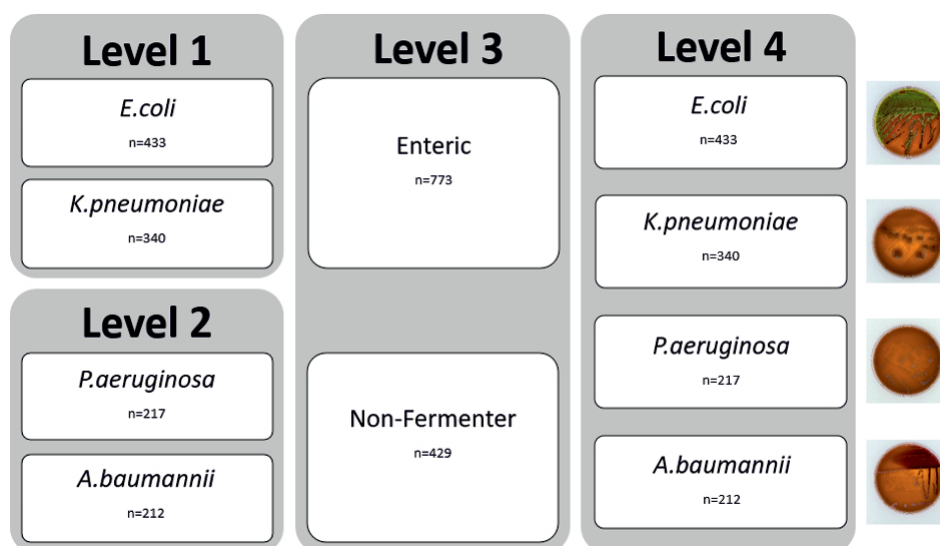


Fig. 1. Classification of bacterial classes

for each class, 20 representative colony images not included in the training and confirmation set were used.

Teachable Machine. In this study, Google's Teachable Machine[®] open AI platform was used with the aim of creating a machine learning network capable of identification from bacterial colony images. Epoch is the hyperparameter expressing the number of samples in the training dataset fed at least once through the training model on this platform. All classes of colony photographs were loaded into the system, separated according to each level (Fig. 1) and the epoch affecting AI performance was set to 50, 100, 150, 200, and 250 with machine training completed ten times each. From the loaded colony images, 85% were randomly chosen for training, while the other 15% were used for confirmation. After training, Teachable Machine[®] created diagnostic network and accuracy for each class. The mean accuracy results for epochs and classes with significantly high performance were assessed with the Mann–Whitney U test. The study received permission from Ordu University clinical research ethics committee (2023/250).

Results. Images of bacterial colonies. A total of 1202 bacterial colony images were used as dataset for training and validation. Level 1 included 433 *E. coli* and 340 *K. pneumoniae*. Level 2 included 217 *P. aeruginosa* and 212 *A. baumannii*. Level 3 included 773 enteric and 429 non-fermenting bacteria. Level 4 separately investigated all of the bacteria. Apart from these, with the aim of assessing the performance of the designed network in Level 4, 80 representative colony images that did not overlap with the training and validation set were used.

Network verification. The mean accuracy according to epoch value for Level 1, Level 2, Level 3 and Level 4 and the hit rates for the classes in the epoch with highest performance are shown in Fig. 2A, B, C, and D. In Level 1, a statistically significant difference was not observed between epochs (Fig. 2Aa). The epoch with highest performance was 250 and mean accuracy was identified as 96.7 ± 1.8 . Mean hit rates were 96.5 ± 3.2 and 96.8 ± 1.7 for *E. coli* and *K. pneumoniae* and no statistical difference was identified between classes (Fig. 2Ab).

In Level 2, a statistically significant difference was not observed between epochs (Fig. 2Ba). The epoch with highest performance was 150 and mean accuracy was identified as 94.1 ± 2.7 . The hit rates for *P. aeruginosa* and *A. baumannii* were 96.1 ± 2.8 and 92.1 ± 4.8 (Fig. 2Bb). The hit rate for *P. aeruginosa* was significantly higher compared to the rate for *A. baumannii* ($p < 0.05$).

In Level 3, epoch 100 had the highest performance (Fig. 2Ca). Mean accuracy was identified as 94.3 ± 1 , which was significantly higher compared to epoch 50 and epoch 250 ($p < 0.05$). The hit rates for enteric and non-fermenting bacteria were 95.1 ± 2 and 93.5 ± 2.3 and there was no statistical difference identified between classes (Fig. 2Cb).

In Level 4, no statistically significant difference was observed between epochs (Fig. 2Da). Epoch 50 had highest performance and mean accuracy was identified as 90.3 ± 2.5 . The hit rates for *E. coli*, *K. pneumoniae*, *P. aeruginosa* and

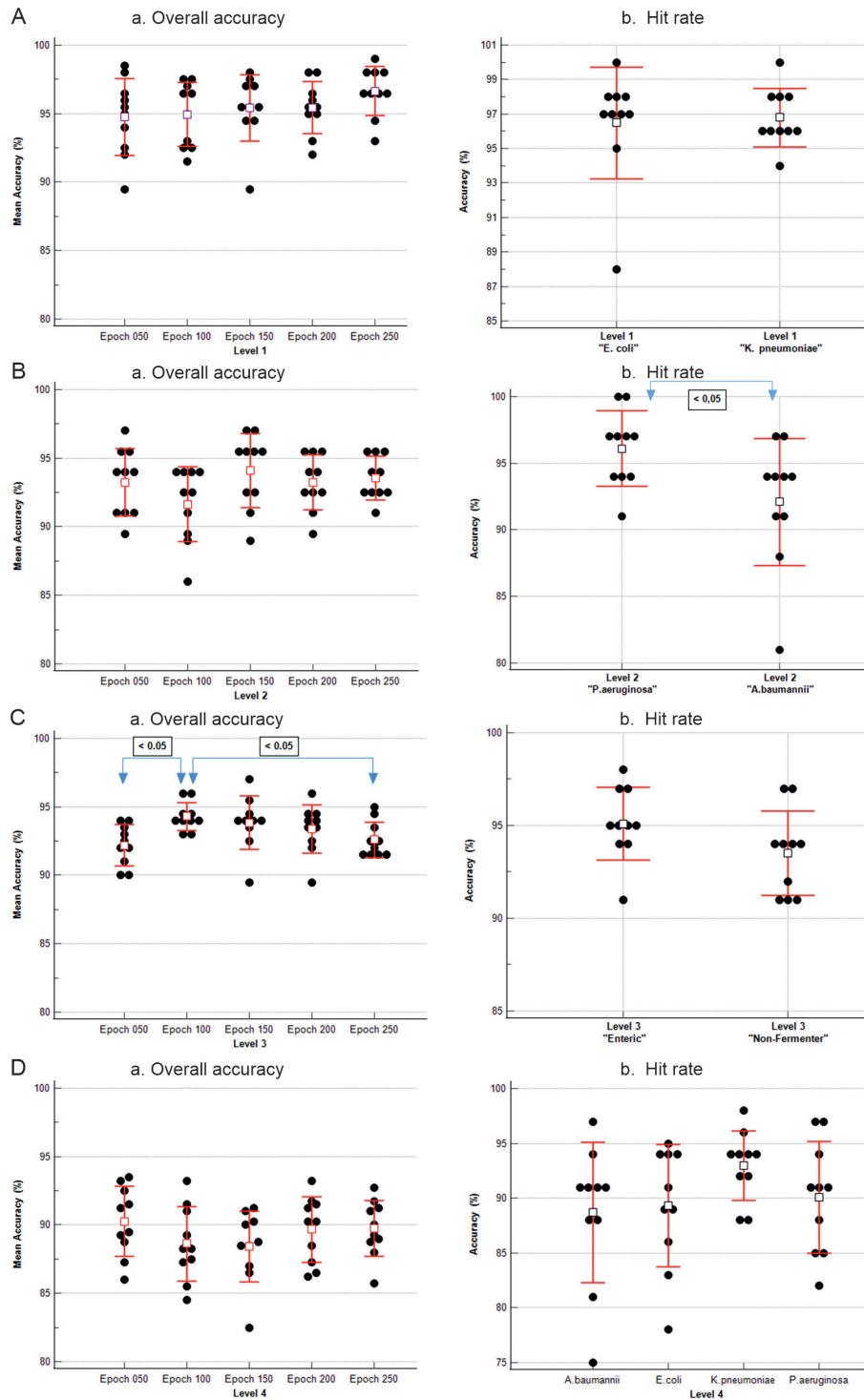


Fig. 2. The mean accuracy according to epoch value for Level 1, Level 2, Level 3, and Level 4, and the hit rates for the classes in the epoch with highest performance

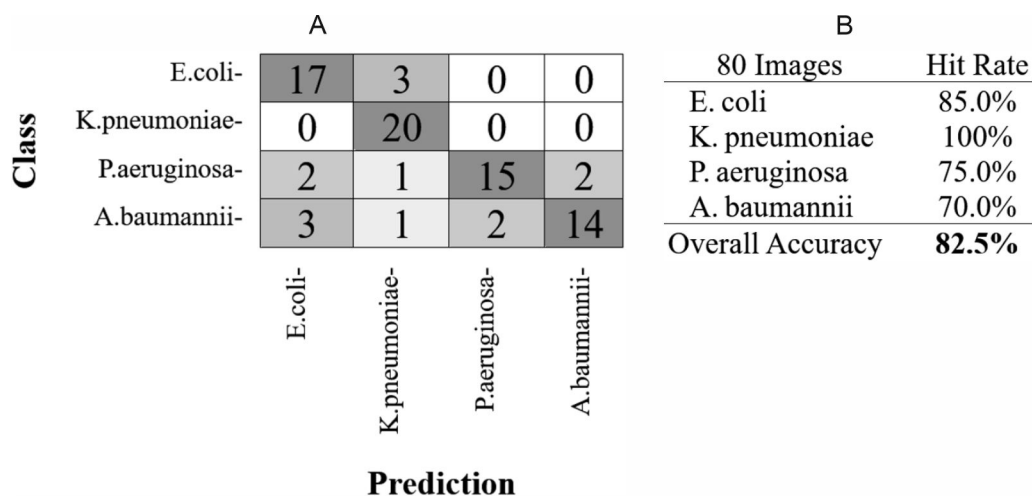


Fig. 3. (A) Confusion matrix for representative images; (B) Overall accuracy of the machine learning network and hit rates for each classification

A. baumannii were 89.3 ± 5.6 , 93 ± 3.2 , 90.1 ± 5.1 and 88.7 ± 6.4 with no statistical difference identified between classes (Fig. 2Db).

Network performance with representative bacterial colony images.

Figure 3A shows the confusion matrix related to the Teachable Machine[®] performance. The developed network achieved 82.5% accuracy rates for performance in identifying the 80 representative bacterial colony images (20 for each class) (Fig. 3B). The hit rate was 85.0% for *E. coli*, 100.0% for *K. pneumoniae*, 75.0% for *P. aeruginosa*, and 70.0% for *A. baumannii*. Three *E. coli* strains were misidentified as *K. pneumoniae* and all *K. pneumoniae* strains were correctly identified. *P. aeruginosa* and *A. baumannii* were confused at higher rates compared to the other classes.

Discussion. In this study, the machine learning platform Teachable Machine[®] was used for identification of the most frequently observed gram-negative bacteria in routine practice and the performance of the developed network was assessed. The findings of our study are as follows: (1) the mean accuracy of epochs with highest performance were 96.7 ± 1.8 for Level 1, 94.1 ± 2.7 for Level 2, 94.3 ± 1 for Level 3, and 90.3 ± 2.5 for Level 4. (2) Mean accuracy for classification of representative bacterial colony images was 82.5%. To the best of our knowledge, this is the first example of an automatic design and application using deep learning model types for bacterial identification applications by people who are not AI experts or bacteriology experts.

In clinical microbiology laboratories, among gram-negative bacteria, strains of the enteric bacteria of *E. coli* and *K. pneumoniae* and non-fermenting bacteria of *P. aeruginosa* and *A. baumannii* are most frequently isolated [10]. In our study, this situation was noted when creating classes. Additionally, these

pathogens are among the antimicrobial resistant ESKAPE pathogens and form a global threat to human health. As a result, rapid identification and treatment is very important [11].

Colony morphology is a defining feature for bacterial species and groups. The physical appearance like size, shape, surface structure and colour of colonies forming on solid medium carry the specific characteristics of the bacterial species. As a result, it is possible to identify bacterial colonies by observing macroscopic morphology. There is lactose within the EMB and bacteria using lactose create dark colonies by acidifying the medium. Additionally, encapsulated bacteria produce mucoid colonies. *E. coli* creates lactose-positive (purple) colonies on the EMB agar surface and additionally, the majority give a metallic green reflection. Encapsulated colonies of *K. pneumoniae* create larger mucoid-moist colonies. *P. aeruginosa* creates R-type and sometimes encapsulated colonies, while *A. baumannii* creates S-type colonies [12, 13]. In light of this knowledge, microbiology professionals perform advanced identification by moving from this information obtained in the first step of identification.

There are device systems performing identification according to the biochemical features of the microorganism. In addition to these, expensive devices in molecular methods and MALDI-TOF may be used [14]. Inexpensive techniques are time-consuming, while systems performing identification in a short duration are expensive. Additionally, there is a need for an experienced laboratory expert to apply and assess all these processes.

AI studies are used for purposes like prediction, classification and clustering, in addition to providing products for different areas of daily life [15]. The Google's Teachable Machine[®] application working within the browser offers a machine learning model and is notable as the easiest tool for those without coding knowledge. Studies in recent times have shown the applicability of Teachable Machine[®] for diagnosis of a variety of diseases [16]. Just as with all supervised machine learning applications, test performance is closely related to the quality and diversity of the training dataset. There are studies using datasets created from available images; however, the appropriate media used routinely in every laboratory and the most frequently observed bacteria are not found in this database [3]. There is equipment to overcome sheen on the plate surface, however, purchasing costs are high. Smith et al. [9] developed a cheap and effective apparatus for high-quality imaging of microbial colonies. We built this apparatus and trained AI by taking colony photographs meeting the same standards.

In a variety of studies, it was shown that bacterial identification could be performed using colony images and AI. The identification accuracy varied from 75% to 99% according to the bacterial species investigated and the AI used [17]. In our study, the mean accuracy was 82.5%. There is no other study in the literature assessing the bacterial identification capability of AI by uploading photographs taken with a simple and cheap-to-produce diffuser and mobile phone camera to

a simple and free machine learning platform with drag and drop web interface. In this way, identification in the laboratory will become much more practical. Additionally, after machine learning with this system, live colony identification can be performed with a phone camera and the Teachable Machine® mobile application. This reasonable cost bacterial identification system supported by deep learning may be a pioneer for very diverse applications in microbiology by significantly reducing the identification duration and automating identification of colonies without requiring a specialist.

Every development in technology or any innovation in science always aims to facilitate and improve human life. Considering the results of this study, the proposed technique may accelerate the identification process for gram-negative bacteria most frequently observed in laboratories. The use of AI for bacterial identification analyses will lead to an increase in efficiency and accuracy. It appears unavoidable that the number of AI tools, quality and reliability of analyses completed by AI software and integration of AI into the workflow of clinical microbiology laboratories will increase. As a result, we believe AI will be beneficial for clinical microbiology practice in future years. However, it should not be forgotten that identification of bacterial species cannot be performed only based on morphological features. Analyses based on genetic structure or laboratory tests are required. For high accuracy rates with AI identification of bacterial colonies, it is still necessary to use biochemical and molecular analyses.

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