Classification and Detection of Pathogens from Enhanced Microscopic Images

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ABSTRACT

Drinking water is essential for human life but unfortunately every year, many lives are lost as a result of the use of polluted water. Computerized methods play a dynamic role in detecting pathogens from water. The first symptoms of pathogens in water are difficult to detect by the naked eye at an early stage. Therefore, in this research, a computerized method is proposed in which features are extracted from the pre-trained ResNet-50 model, and classification of the different types of pathogens is performed using the SoftMax layer. The proposed method's performance is evaluated on the proposed microscopic pathogen dataset. The proposed dataset is pre-processed using Enhanced Super-Resolution Generative Adversarial Networks (ESRGAN) method for the enhancement of Image quality. The proposed method provides greater than 90% prediction accuracy.

Keywords: *Image Acquisition, Pre-Processing, Features extraction, Segmentation, Super Resolution*

1. INTRODUCTION

A pathogen is characterized as a living being making an infection on its host, with the seriousness of the sickness manifestations alluded to as virulence. Microbes are methodically comprehensively different and incorporate contaminations and infinitesimal creatures similarly to unicellular and multicellular eukaryotes. Each living organic entity is influenced by the pathogen, including microbes, which are focused on particular infections called phages [1]. A microbe is ordinarily portrayed as a microorganism that causes or can cause the disorder. A microbe is described as an organic entity that can cause hurt to a host. Different types of pathogens are bacteria, viruses, fungi, etc. [2].

One of the key elective recognition methods being studied is polymerase chain response (PCR). PCR is a quick, very sensitive, and precise method. It has been used to distinguish infectious illnesses, microorganisms, and protozoa in water [3]. Water is essential at all times. In any case, various people do not advocate for the provision of high-quality, clean drinking water, and bacterial pollution of water is a major cause of death for many people. One of the most important challenges of the twenty-first century, it was considered, is providing everyone with access to clean water, and that the global norm for drinking water should be microbiological control [4]. The majority of financial resources should go toward increasing understanding of the science and factors that influence the existence of human and animal waste in rivers.

The majority of water sensors are chemically based, and most water testing procedures are now conventional. Chemical test strips, which are one-time use, are the most often used approach. Contamination monitoring is a challenging and time-consuming process. Water AI is an Internet of Things (IoT) gadget that identifies and detects viruses in water [5]. Water and pollution are constantly monitored by this equipment. They collect data from cameras and other edge devices, then analyze it to discover and classify hazardous microorganisms. It is necessary to monitor water resources and analyze water quality regularly to protect humans from water-borne illnesses caused by bacteria, viruses, and protozoa. Consumers expect non-toxic drinking water that satisfies quality requirements and is pleasing to the eye in terms of color, taste, and odor.

Some researchers also used image-processing-based techniques for the classification and detection of foodborne and waterborne bacteria to make the process of detecting contamination faster and more accurate. As [6] proposed a convolution neural network (CNN) technique to categorize the microorganisms Vibrio cholera (V. cholera) and Escherichia coli (E. coli) in wastewater. A software CellC is also developed to perform automatic image analysis and measure the number of bacterial cells in images from a digital microscope [7]. A Neural Network-Based Image Analysis model is also proposed in [8] to rapidly determine the Abundance, Biovolume, Morphology, and Growth of bacteria. But still, these methods aren't up to the mark and there is a need to develop an automatic method for the accurate detection and classification of bacteria to save many precious human lives.

2. RELATED WORK

This section will discuss some existing work performed by different researchers to detect and classify pathogens from contaminated water. Individuals from the Vibrio class are autochthonous occupants of sea-going conditions and assume imperative parts in supporting the sea-going milieu [9]. The variety consists of over 100 species, the majority of which are freshwater or marine roots, and their classification is often updated as new species are continuously discovered. Vibrio diseases are transmitted to humans mostly by the consumption of polluted water and the consumption of improperly prepared sea foods. Some rural tenants in Sub-Saharan Africa and a huge portion of the developing scene utilize freshwater assets such as streams for indigenous workouts, washing, and social and strict functions. The danger to the province's population who depend on the water supply is shown in this audit, which also shows the effects of poorly treated sewage effluents on the province's ability to acquire freshwater assets [10]. Contaminations with vibrio continue to be a danger to the general public's health. Vibrio infection outbreaks have heightened the individual, financial, and general well-being risks associated with the impact of degraded water in Sub-Saharan Africa's oceanic climate in the last decade. To aid in the reduction of risk associated with the early discovery of contamination sources and to better understand the usual nature and course of Vibrio illnesses, consistent monitoring of Vibrio bacteria in biological freshwater and treated effluents is crucial. [11]. Waterborne pathogens represent a huge danger to human well-being and an appropriate appraisal of microbial water quality is significant for dynamics in regards to water foundation and treatment speculations and at last to give early notice of infection, especially given expanding worldwide debacles related to extreme general well-being hazards. Microbial water quality checking has gone through a gigantic change lately, with novel atomic instruments starting to offer fast, high-throughput, touchy and explicit identification of a wide range of microbial pathogens that challenge conventional culture-based procedures [12]. Microbial pathogens are one of the significant well-being hazards related to water and wastewater. Current strategies for the discovery of pathogenic infections, microorganisms, protozoa, and Helminthes in general be erroneous, tedious, and costly. Subsequently, marker microscopic organisms are regularly used to decide the general danger of fecal defilement and the conceivable presence of pathogens in water and wastewater. Pointer creatures, nonetheless, have a few weaknesses that make them not exactly ideal for showing the conceivable presence of microbial pathogens. Thusly strategies to straightforwardly distinguish microbial microorganisms in water and wastewater are being explored [13]. Checking the event and thickness of parasites and pathogens can recognize high contamination hazard territories and encourages infectious prevention and destruction measures. Ecological DNA (eDNA) procedures are progressively utilized for microbe identification because of their overall simplicity of utilization. Since numerous variables influence the unwavering quality and adequacy of eDNA-based recognition, thorough approval and appraisal of strategy restrictions is a pivotal initial step. We assessed an eDNA location strategy utilizing in situ filtrations of enormous volume water tests, created to recognize and measure oceanic untamed life parasites by quantitative PCR [14]. To screen viral, bacterial, and protozoan microbes, as well as to track microorganisms and source-explicit indicators in the climate, atomic methods are used. Sub-atomic methodologies, including polymerase chain response-based approaches, provide sensitive, fast, and quantitative scientific instruments for concentrating microorganisms, including novel or emerging strains. These techniques are used to evaluate the microbiological composition of food and water, as well as to examine the efficacy of infection expulsion in drinking and wastewater treatment plants [15]. The scope of strategies accessible for the utilization of atomic methods has expanded, and the costs included have fallen.

These improvements have permitted the possible normalization and computerization of specific methods. At times they encourage distinguishing proof, genotyping, count, suitability appraisal, and source-following of human and creature tainting. Also, late enhancements in location advancements have permitted the synchronous identification of different focuses in a solitary measure [16, 17]. A method for analyzing the effectiveness of convolutional neural networks for the detection of bacteria in 3D microscopy datasets was proposed in [18]. [19] performed a performance analysis of machine learning methods for classifying images of microscopic bacteria. [20] proposed employing a convolutional neural network with fine-tuning to enhance the detection of gram-negative bacteria. UNet++ is used in [21] to do automatic bacillus anthracis bacterium recognition and segmentation in microscopic images. [22] suggested a morphological image analysis for foodborne bacteria classification.

3. METHODOLOGY

This research work is conducted to identify and classify pathogens from containment water and to provide a solution using a fully automated system. Image capture, pre-processing, and super-resolution for obtaining low and high-frequency information from cell pictures, breakdown of frequencies, and categorization of distinct kinds of bacteria are all phases in the proposed methodology. Fig. 1 presents the Classification of Pathogens (Water Bacteria).

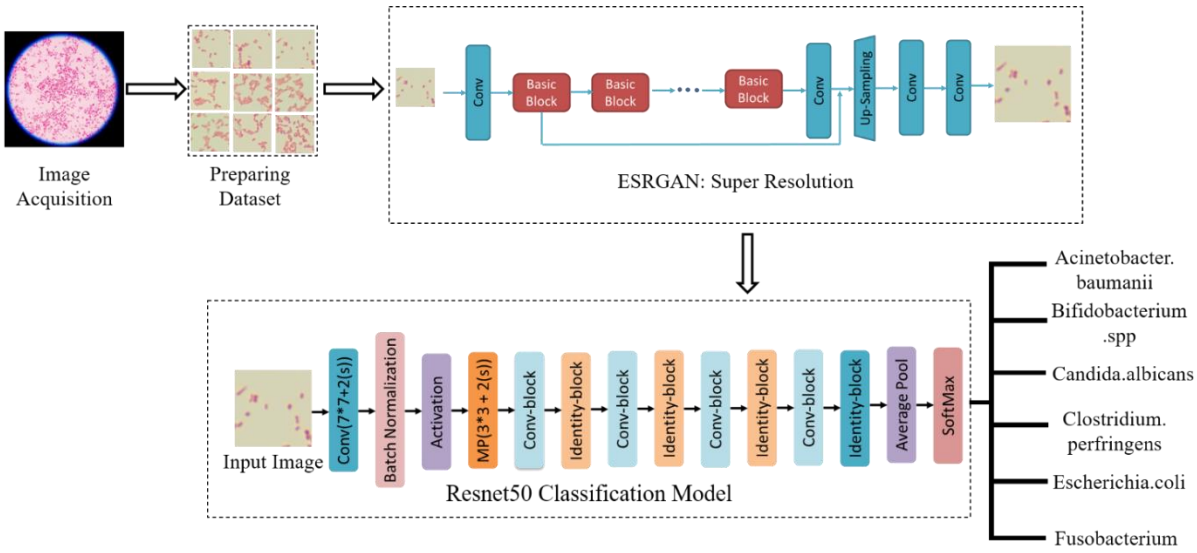


Fig. 1. Proposed Methodology for the Classification of Pathogens (Water Bacteria)

For the grouping of microbes, the resnet50 model was prepared to separate conventional highlights. For classification, a pre-trained model of ResNet-50 is used which consists of 50 layers, and its training was done on a million images from 1000 different categories taken from the ImageNet database. In the testing stage, every cell image is passed to the prepared resnet50 demonstrate and use max-pooling for the forecast. The proposed methodology consists of three steps: 1) Image Acquisition, 2) Preprocessing, and 3) Feature Extraction and Classification. The general flow of the proposed methodology is shown in Fig 1 below. Fig 1 shows the three modules, first is image acquisition in which microscopic images of the pathogen are generated and collected through the gram-staining process. In the second phase, the preprocessing is performed on the image acquired through the gram-stained process. ESRGAN is used in this phase to convert the low-resolution patches (extracted from slides) into high-resolution images. In the third and last phase, ResNet50 is used for feature extraction and classification of different types of pathogens.

3.1 IMAGE ACQUISITION

For image acquisition, we performed experiments on the lab complex, at the University of Wah. Images were collected by using the gram staining process. The E.coli images collected from the lab are shown in Fig. 2.

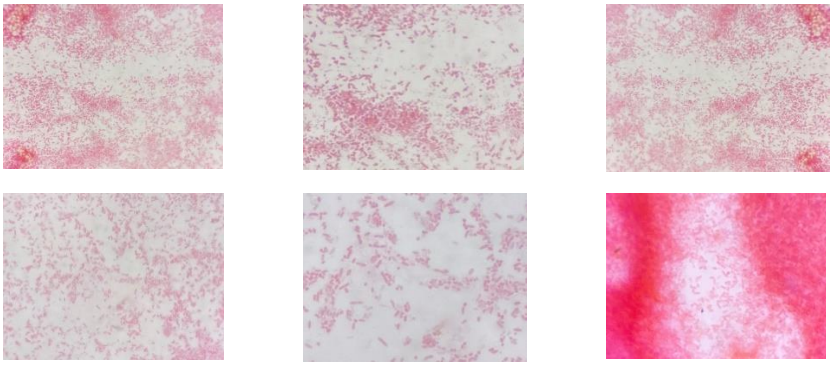


Fig. 2. Samples of Slide Images (Gram Staining) Collected in Lab Complex, University of Wah

3.2 PREPARATION OF THE SMEAR

The first step in most bacterial staining procedures is the preparation of a smear. For several laboratory procedures, including the Gram stain, a smear must be prepared. Using methanol or warmth, adhere the material to a slide. Allow the slide to cool to the touch before staining it.

3.3 PROCEDURE FOR GRAM STAINING

Following are procedure for gram staining

- Firstly, for 1 second, an air-dried, heat-fixed smear of cells is dipped in a gem violet staining reagent.
- After that, the slide is streamed with a gentle, backhanded spray of faucet water for 2 seconds.
- Then, gram's iodine the most stringent is used to flood the slide.
- After that for 2 seconds, the slide is washed in a gentle, roundabout stream of tap water.
- In the next step, the slide was decolorized with a decolorizing expert (Acetone-liquor decolorizer).
- The slide is stained with a counterstain, which is followed by a gentle, circular stream of tap water washing it off for 30 to 1 minute or until no more counterstain is visible on the slide, and finally blotting dry with absorbent paper.
- In the last step, using a bright field magnifying tool, the aftereffects of the staining technique are observed under oil inundation (100x).

3.4 DATASET PREPROCESSING

Data preprocessing is a term used to describe the process of cleaning and organizing raw data to make it suitable for the creation and training of classification models. The slides generated through gram staining for the local datasets were clustered with numbers of pathogens so as a first preprocessing step, the slides are divided into multiple patches. The visual representation of the different types of bacteria images is shown in Fig 3.

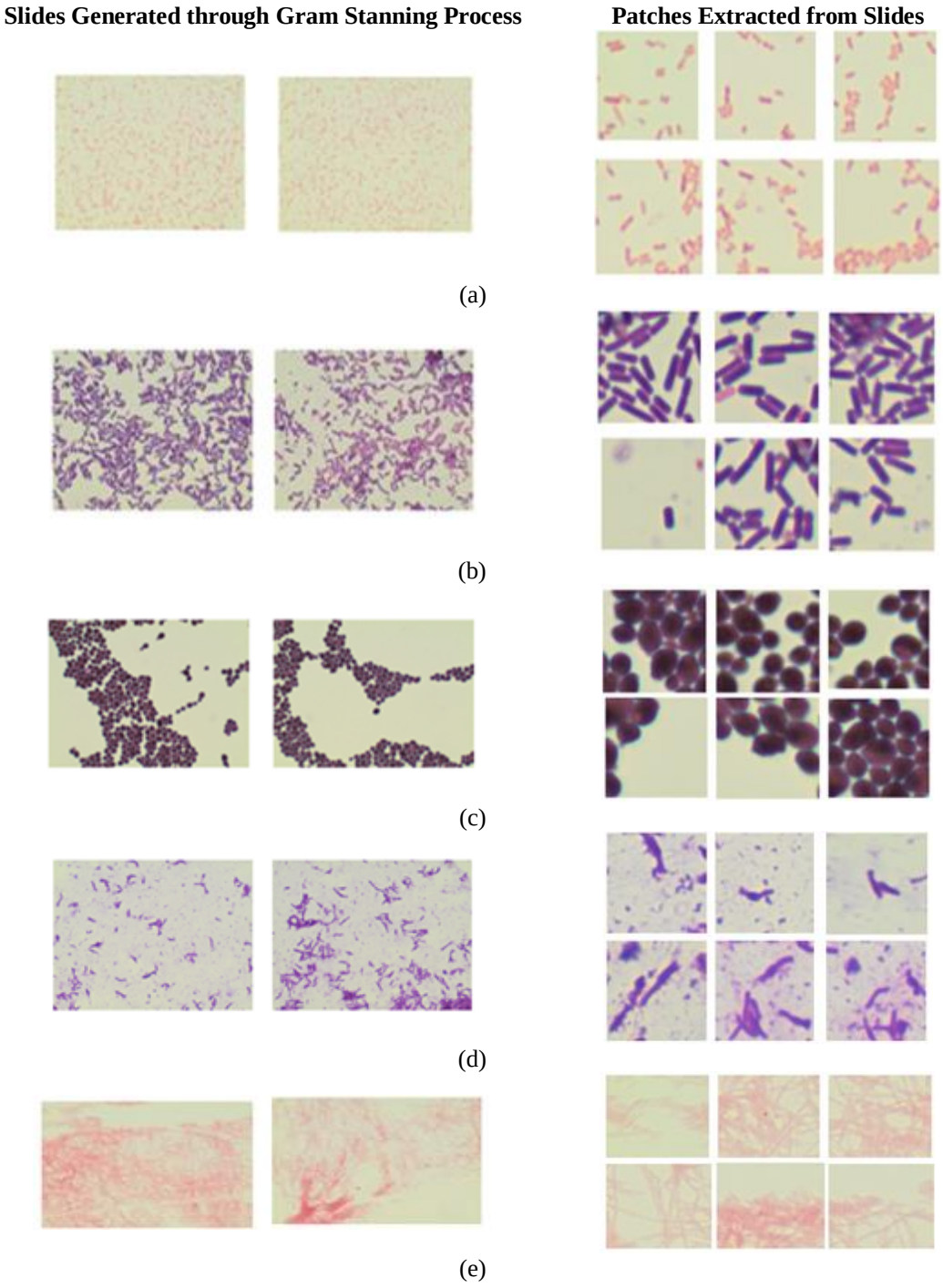


Fig.3. Generating Dataset Images from Slides: (a) Acinetobacter.baumanii (b)Clostridium.perfringens (c) Candida.albicans (d) Bifidobacterium.spp (e) Fusobacterium

3.5 SUPER RESOLUTION

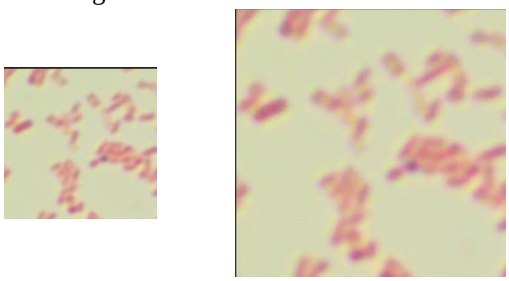
The primary objective of the super-resolution is to produce a high-resolution image from one that already has a low resolution. Super-resolution enhances the resolution and quality of an image. The patches of our dataset were not clear and the qualities of the images were very low so, a super-resolution process is employed to improve the image's quality, taking it from a low resolution to a high resolution. ESRGAN [23] is used for this task which used GAN (Generative Adversarial Network) for performing single image super-resolution.

Network interpolation is an approach that is both flexible and successful. It is employed in GAN-based methods to eliminate unwanted noise while still keeping a high level of perceptual quality. In particular, a trained PSNR-oriented network known as Gpsnr, followed by the acquisition of a GAN-based network known as GgAN via fine-tuning is used for enhancing the images. Then in this step to interpolate all of the parameters that belong to these two networks to produce an interpolated model called Gin. The parameters of this model are as follows:

$$\theta_g^{in} = (1 - \alpha)\theta_g^{psnr} + \alpha\theta_g^{gan} \quad (1)$$

where (0; 1] is the interpolation parameter, θ_g^{in} is the parameter of Gin, θ_g^{psnr} is the parameter of Gpsnr, and θ_g^{gan} is the parameter of Ggan, respectively.

There are two benefits to the suggested network interpolation. To begin, the interpolated model may reliably, and without artifacts, provide meaningful results for any viable. Second, we don't need to re-train the model every time we want to strike a compromise between perceived quality and fidelity. The results of applying ESRGAN on images are shown in Fig 4 below:



(a) (b)

Fig.4. Super-Resolution Applied using the ESRGAN Method: (a) Before (b) After [18]

3.6 CLASSIFICATION

For the classification of pathogens, transfer learning is used. A pre-trained ResNet50 model [24] is utilized which is a 50 layers neural network in which training was done on a million images from 1000 different categories taken from the ImageNet database. The training process was carried out. The model that is used for the pre-training consists of five stages, and each step includes a residual block that is included in it. Each residual block is composed of three layers and features convolution filters arranged in a 1*1 and 3*3 configuration. The concept of blocks that were not used is not too complicated. A network that contains residual blocks has one layer that feeds into the next layer and straight into the levels that are around two to three hops distant from it. Identity connections are the name given to these types of connections. For the classification purpose, the enhanced images of pathogens are passed to the ResNet50 model, and images are categorized into 6 classes.

4 RESULTS AND DISCUSSION

The original dataset was gathered from the lab of the Bioscience department in Lab Complex, University of Wah combined with the DIABs dataset. The gram standing process is used for the local dataset for E. coli images. DIABs dataset has 33 classes but the proposed methodology used only 6 classes for the training and testing and each class contains 20 bacteria images. The dataset was expanded from 20 to a minimum of 1080 bacteria images per class. The model used 5573 images for training from the total images of 6966 and the remaining images are used for testing and validation. The given paragraphs show the result of different stages during classification. The proposed method results are mentioned in Tables 1 and 2.

Table 1. Training Results of the Proposed Method

Epochs	Training Loss	Validation Loss	Accuracy	Time
1	0.392279	0.158943	0.961953	01:49
2	0.257867	0.085959	0.968413	01:18
3	0.229803	0.063270	0.979182	01:17
4	0.135165	0.044037	0.984925	01:18
5	0.113938	0.047994	0.981335	01:17

The proposed model was trained on five epochs as shown in table 1. The table shows that the Training Loss on the fifth epoch is 11%, with a validation loss 0f 0.47% and an accuracy of 98%. The training time of the model is 1 minute and 17 second.

Table 2. Testing Results of the Proposed Method

Epochs	Training Loss	Validation Loss	Accuracy	Time
1	0.165309	0.070150	0.976310	01:28
2	0.188746	0.200356	0.926059	01:28
3	0.214985	0.089699	0.971285	01:27
4	0.195290	0.173984	0.870065	01:25
5	0.155426	0.298054	0.981335	01:27
6	0.082419	0.565252	0.970567	01:27
7	0.065206	0.926246	0.961235	01:28
8	0.022595	0.299336	0.969131	01:28
9	0.022068	0.468280	0.969849	01:29
10	0.013831	0.761713	0.953338	01:27

The learning of the proposed model is on 10 epochs as shown in table 2. The table shows that the Training Loss on the tenth epoch is 1%, with an accuracy of 95%. The training time of the model is 1 minute and 27 seconds.

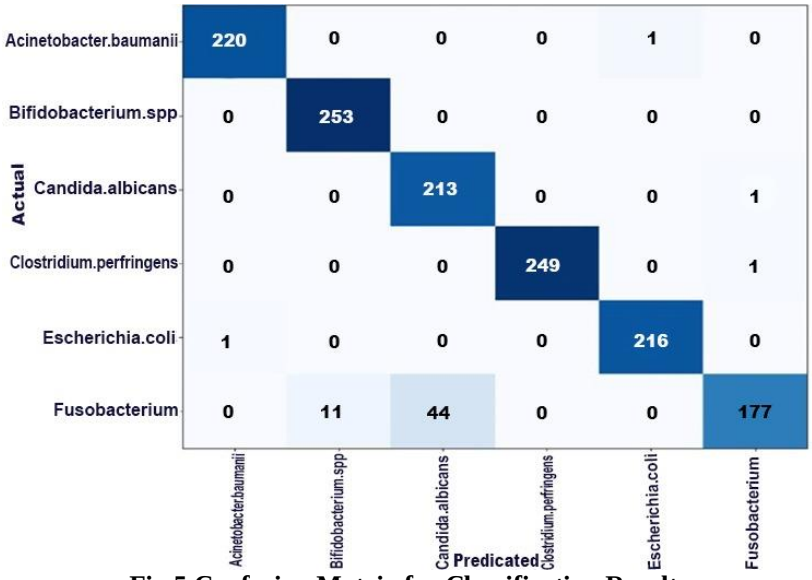


Fig.5 Confusion Matrix for Classification Results

The classification results for the 6 categories of the pathogen are shown in fig.5 and the graphical representation of the confusion metric for the classification is shown in Fig.6. The results show that the highest accuracy is achieved against Bifidobacterium. spp which is 100%.

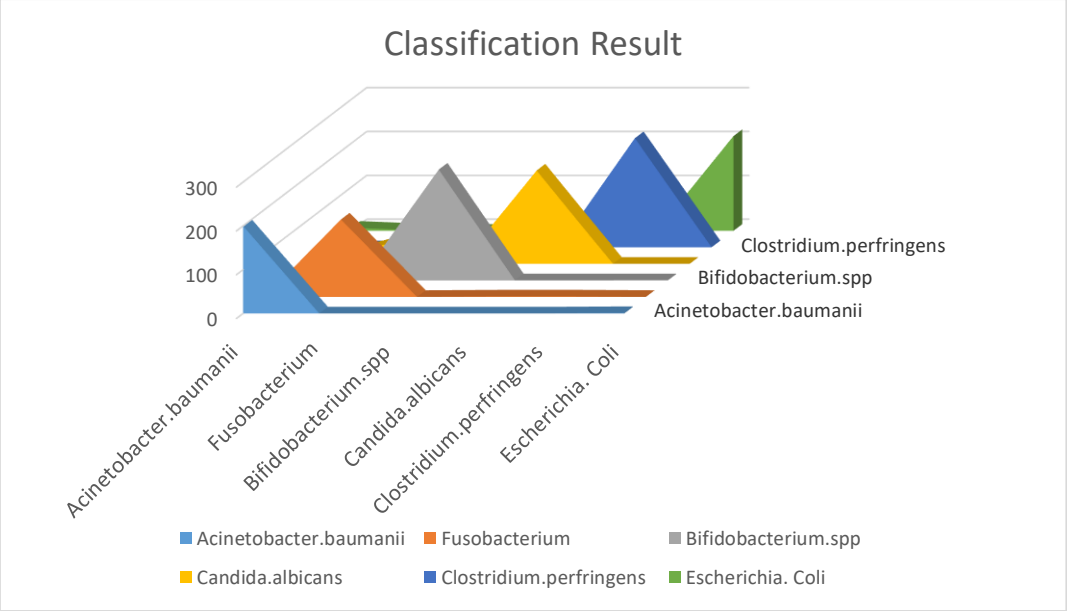
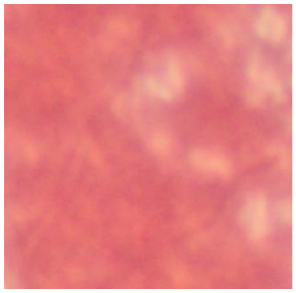
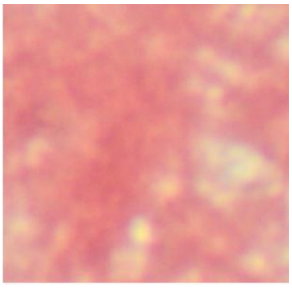


Fig. 6. Graphical Representation of Classification Results on the Dataset

The probability and loss of the proposed model are shown in Fig 7. In Fig 7, the prediction is compared with the actual class and its loss and probability are shown with the predicted and actual labels of the class. The Fig shows that the loss against Bifidobacterium.spp against Fusobacterium is 49.72, 44.84, and 42.61 and for Candida.albicans against Fusobacterium it is 40.94.



Bifidobacterium.spp/Fusobacterium/
49.72/0.00
(a)



Bifidobacterium.spp/Fusobacterium/
44.84/0.00
(b)

Bifidobacterium.spp/Fusobacterium/
42.61/0.00
(c)

Candida.albicans/Fusobacterium/
40.94/0.00
(d)

Fig. 7. Sample Results for the Probability and Loss of the Model(a-d)

5 CONCLUSION

This research paper provides a fully automated System sin which easily detects pathogens at the early stages. Image processing techniques are used to detect the pathogens and ResNet50 is used for the classification of pathogens. There are numerous difficulties in the automatic processing of cytological images of bacteria. The main problems include a large variation of microorganisms, low image quality, and a lack of real-world data. In this work, these issues are addressed. This work presents a method for classifying microscopic bacteria images systematically and effectively. Our system's classification and accuracy with normal samples are good, according to the findings of our experiments. In this work, a framework divided into three main stages: image pre-processing, super-resolution, and classification is proposed. with good recognition accuracy even in presence of low-resolution images and noise. It achieves 95% accuracy in the learning model and 98% accuracy in the training model.

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