Drinking Water Monitoring: Computer Vision Kit for Early E. Coli Detection

Mansoor Khan[1], Samad Riaz[2], Gul Muhammad Khan[2]
[1]Department of CS & IT UET Peshawar, Pakistan
[2]Department of Electrical Engineering UET Peshawar, Pakistan
*Correspondence: mansoor.khan@uetpeshawar.edu.pk, samadriaz@uetpeshawar.edu.pk, gk502@uetpeshawar.edu.pk

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This work presents an easy-to-use and accurate method to find up to 1 coliform unit (CFU) of a pathogenic bacterium i.e., Escherichia coli (E. coli) in 100ml of drinking water in 6-8 Hours of the incubation period. A larger number of CFUs is easy to detect and incubation time is reduced to 5-7 Hours for the testing samples containing more than 20 CFUs. Normally in laboratories up to 1 ml of a water sample is spread on an endo agar medium and incubated for about 24 Hours, and the E. coli coliform in metallic green color becomes visible through the naked eye. Which has a limitation of finding 1 CFU in just 1 ml of water and a limitation of a large amount of time. In the proposed work Membrane filtration method is used for experiments and a microscopic camera with deep learning algorithms i.e., yolov5 and yolov8 is used for the early detection and counting of E. coli colonies. This system is generalized on the field data of 8k images taken from different cities' water samples in Pakistan. Yolov5s model achieved a mean average precession (mAP@0.5) of .949, while the latest release version yolov8 achieved mAP@0.5 of 0.950. An automatic imagery system is developed that takes the images just by placing a petri dish in it processes those images through Raspberry Pi, and shows the detected colonies on the screen, while remote users can use a low-cost microscopic camera manually with a developed mobile application.

Keywords: Water Quality; Escherichia coli; Computer Vision.

Introduction:

Water contamination is a global health issue. According to the World Health Organization (WHO), at least 1.7 billion people worldwide rely on contaminated drinking water. Microbial-contaminated water serves as a transmission source for various pathogens, including bacteria and harmful chemicals, and poses the greatest risk to drinking water safety. It can transmit diseases such as diarrhea, cholera, dysentery, typhoid, polio, and a range of digestive, respiratory, and neurological issues. It is estimated to cause approximately 505,000 diarrheal deaths each year [1]. Therefore, safe drinking water is essential for protecting public health and reducing the risk of waterborne diseases. To ensure its safety, drinking water must be free from harmful levels of pathogens and contaminants. E. coli is a type of bacteria that is commonly found in the environment, including in soil, water, and the intestinal tracts of animals and humans. Some strains of E. coli can cause illness in humans, particularly when they are present in drinking water. E. coli can enter drinking water sources through the feces of infected animals or humans [2]. Escherichia coli (E. Coli) pathologies are classified based on the ailments it causes. Each pathotype results in a unique set of illness symptoms. E. coli O157:H7 is a strain of E. coli that produces a toxic substance called Vero toxin or Shiga toxin, which can damage the lining of the small intestine and cause severe diarrhea [3]. In light of illness causes, it is crucial to quickly and accurately identify E. coli bacteria to stop epidemics and lower the death rate.

One common method for detecting E. coli bacteria is the use of agar media, which is a solid growth medium that contains nutrients and other components that are necessary for the growth and metabolism of microorganisms [4]. This method is limited by time, energy, human error, and testing of very small water samples. However, this method can be used for the creation, isolation, identification, counting, and sensitivity testing of microorganisms, as well as the testing of clinical specimens, food, water, and environmental controls. Deep learning approaches like convolution Neural Networks (CNN) can learn complex patterns in the data and play important roles in measuring the concentration of cyanobacteria in water detecting water impurities [5], etc. In [6] CNN is used with microscopic images for the classification of E. coli and Vibrio cholera (V. cholera) in water waste which usually contains a large number of bacteria and the dataset is collected in the laboratory with a high-resolution microscope from the wastewater samples. In [7] a faster-RCNN algorithm is used for the detection of E. coli bacteria on an endo agar medium using a simple camera in 6-10H, which is useful for the laboratory's experimental purposes. This is limited by the testing very small testing water poured on the solid agar medium, and the culture color change to dark which shows the presence of E. coli, but it doesn't measure the concentration or number of E. coli CFUs present in the testing water sample.

The proposed system uses the membrane filtration method in which 100ml of a water sample is passed from the Millipore membrane filter using a simple filtration assembly and then placed the membrane filter is on an absorbent pad containing methylene lauryl sulfate broth (MLSB) and kept in the incubator. This method aims to find out 1 CFU in 100ml of drinking water. Data is collected both manually and by using an automatic imagery system, from the samples taken from various cities in Pakistan to make a well-generalized dataset of about 33k images. A low-cost microscopic camera is used on which a specific pattern is defined for capturing images manually. Following this pattern while taking images helps in finding colonies that grow anywhere in the petri dish. This is helpful for the remote users using our developed system who have no access to our automatic kit. YOLO is a computationally efficient and accurate framework for object detection unlike two-stage detectors like RCNN, faster-RCNN does object detection in a single pass [8]. A state-of-the-art computer vision model yolov8 is used for the detection and counting of colonies, which is generalized on our dataset. And finally, both the automatic offline and manual online systems are developed in which a trained model is deployed. The automatic kit contains a Raspberry Pi for image processing, a servo motor for

moving the petri dish, and a microscopic camera installed for capturing images from the whole area of the petri dish automatically.

Literature Review:

Numerous studies have been conducted in this field using various methodologies and datasets of images to train their models. The process for the rapid detection of e. coli along with a mobile application is automated and used in [9] by a convolutional neural network (CNN). The CNN model achieved a high accuracy of 96% and was able to predict each sample in just 458ms. The overall process takes 12 to 24 hours and is limited by information on the number of CFUs present in the sample. In [10] a CNN was used to classify and count E. coli and Vibrio cholera (V. cholera) bacteria in wastewater from microscopic images. The CNN had an accuracy of 93.01% and 97.0% for classification and counting, with better performance for the RGB color model. Sensitivity analysis showed that adding Gaussian noise to the images decreased the accuracy of the CNN. A deep learning method (Faster RCNN) using the TensorFlow framework that is 99% accurate and is based on the color variations between images was able to reduce the detection time of E. coli bacteria to 6-10 hours [11]. This is limited by counting CFUs and testing a very small testing sample of pure E. coli in the laboratory because the color variation occurs with other bacteria presence as well. Further classification of bacterial growth in agar plates was carried out with Coherent microscopy and deep neural networks [10]. This system detection can detect up to 1 CFU per 1000ml (1CFU/L) in 9 hours. This system is complex and the experimental setup is expensive, not portable, and more resources consumable. Two different media two different times before and after incubation, manual filtration process after 5 hours, keeping again another media, and then in the imagery system consumes one expert time while experimenting.

R. Patil et al. (2020) detected viable bacterial cells in water samples within a period of 2 hours with LOD of 1-10 CFU/ml using a cell splitting method and developed a neural network-based system that uses time-lapse microscope images with the microscope (Labomed Lx 300i) to detect and quantify viable bacterial cells in water samples [12]. A ResNet50 is used to detect E. coli in images of the optical microscope of water samples collected by lay community workers using a mobile app and field protocols [13]. While the field protocols and mobile app were successful and received positive feedback, the images generated by a low-cost microscope in field conditions were not of sufficient quality for AI detection. The preliminary AI algorithm performed with 94% accuracy in identifying E. coli in lab-derived images compared to a gold-standard method, and additional low-cost technologies are being explored to improve image quality. The Correlation parameters, with the help of artificial intelligence, can accurately detect E. coli in water samples in a short time [14]. Neural network-based method for identifying E. coli in groundwater samples utilizing physio-chemical water quality factors. In [15] titration and spectroscopic techniques were used to examine the water samples for any physical, chemical, or microbial changes. An artificial neural network (ANN) was used to predict E. coli levels in groundwater based on water quality parameters. The best-performing model included Turbidity, pH, Total Dissolved Salts, and Electrical Conductivity as inputs and was optimized using a Bayesian Regularization training algorithm. The superposition-based learning algorithm (SLA) based on Grover's algorithm was effective in accurately predicting E. coli levels and could potentially automate real-time bacterial monitoring. L. Lechowicz et al. (2013) used Infrared spectra to classify 109 uropathogenic E. coli strains based on their susceptibility/resistance to cephalothin using ANN [16]. Bacteria strains were cultured on LB agar medium at 37°C for 24 hours before IR spectra measurement. The best-designed ANN achieved an error rate of 5% and an accuracy of 83.43% in classifying the strains. Infrared spectroscopy and ANN can be used to classify bacteria based on their antibiotic susceptibility. V. Chandramouli et al. (2020) neural network model was developed to predict E. coli levels at six select Lake Michigan beaches using water quality observations and tributary discharge data as inputs [17]. An Excel sheet tool

was developed based on the best model to facilitate real-time decision-making by beach managers. The model, developed using historical data and the Bayesian Regularization Neural Network training algorithm, had an average prediction accuracy of 87% in predicting E. coli classes. M. Stocker et al. (2022) Several machine learning models were evaluated for predicting E. coli concentrations in agricultural pond waters in Maryland over three years [18]. The random forest model provided the lowest root mean squared error in almost all cases and important predictors of E. coli included turbidity, dissolved organic matter content, specific conductance, chlorophyll concentration, and temperature, all the process requires 2 hours at 37℃. Model performance did not significantly differ when using 5, 8, or 12 predictors, indicating that additional measurements did not significantly improve the predictive accuracy of the evaluated algorithms. support vector machines, k-nearest neighbor, and stochastic gradient boosting models also performed well in predicting E. coli concentrations. An artificial intelligence-based system for quasi-real-time water quality monitoring, specifically focusing on detecting early chemical or bio-contamination [19]. The E. coli grow for 1 - 4 hours and the accuracy of the model depends on the time for which E. coli grow. The system used advanced pattern recognition algorithms such as Support Vector Machines (SVM) and ANN, as well as innovative sensing technology, to identify anomalies in the water quality parameters of free chlorine concentration, pH, alkalinity, and total organic carbon. In [20] rapid E. coli detection method using membrane lauryl sulfate broth (MLSB) employs an indirect impedance technique. MLSB medium is prepared, inoculated with water samples or bacterial strains, and sealed in glass cells for 24-hour incubation at 44°C, producing characteristic impedance patterns for detection.

Methodology:

The methodology of the system can be explained in 4 steps i.e., choosing a method for water testing, data collection, model training, and deployment.

Method For Water Testing:

Various kits are used to test drinking water in the field during different emergency cases**.** In [21], three kits are compared i.e., Delagua, Colilert, and Petrifilm based on the accuracy, experimental process, and cost. According to this paper, during an emergency, Colilert MPN should be considered first next to Petrifilm, and last the Delagua, because one can incubate the sample from the human body in the Coliert and Petrifilm kits. On the other hand, based on the accuracy, the Delagua kit is the most accurate of the three, followed by Colilert and Petrifilm. Figure 1 shows the performance of the three methods on different numbers of CFUs. To make an accurate system a Delagua kit methodology is used for water testing in which the water sample is passed through a Millipore membrane filter shown in Figure 2 and placed on the absorbent pad containing media and then placed in the incubator for incubation.

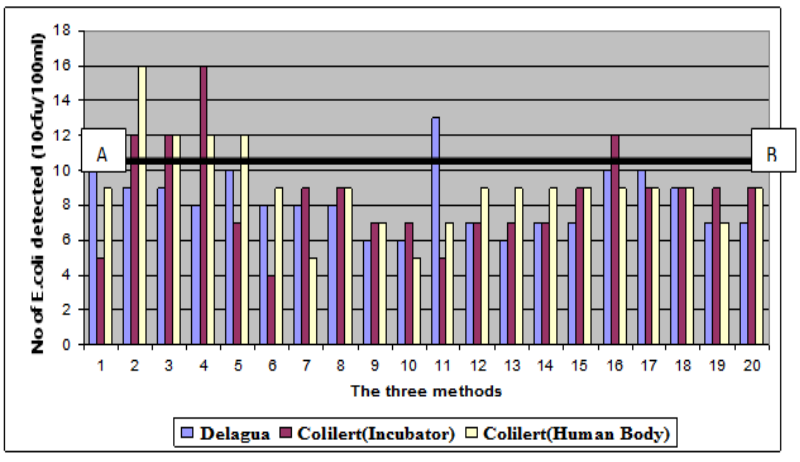

Figure 1: Graphical comparison of results from the three methods at low-level (3rd) dilutions [21]

Setup For Data Preparation:

The deep learning model required a large amount of well-generalized data to learn. Collecting a sufficient amount of data through a microscopic camera is hard, time-consuming, and less efficient. The main challenge is the microscopic camera can't cover the whole area of the petri dish in a single image. It is possible to cover the whole area either by moving the microscopic camera or the petri dish. We have made two setups, automatic and manual for moving the petri dish because moving the camera leads to blur, loss of focus, and moving effects in the images.

Automatic System for Capturing Images:

A simple automatic 2D motion system is designed to move the petri dish both horizontally and vertically shown in Figure 3. It smoothly moves the Petri in front of the microscopic camera without losing the focus on the area. This design is implemented in the final developed prototype shown in Figure 6.

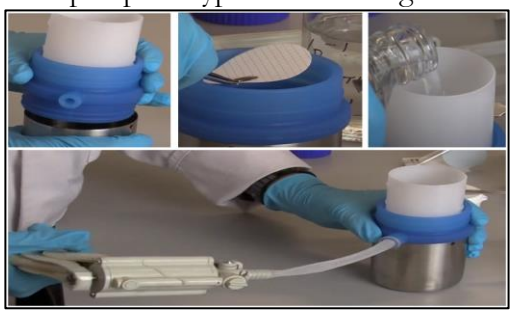

Figure 2: Filtration Assembly Setup, and Method of Using

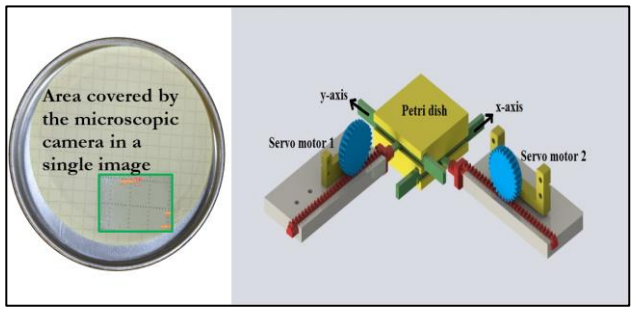

Figure 3: Automatic Moving Petri Dish System Implemented in the Automatic Kit

Figure 4: Manual Setup for Data Collection

Manual System for Capturing Images:

Some data is also collected manually using a 2-megapixel microscopic camera with 50-1000x optical zoom and 1920x1080P resolution shown in Figure 4. The camera comes with a stand, and we paste a custom pattern on the base, following that pattern while taking images through the microscopic camera in a mobile application resulting in covering the whole area of the petri dish in 28 images.

Water Samples for Data Collection:

The drinking water for testing and creating the dataset has been collected from universities, colleges, homes, and restaurants in the cities of Peshawar, Nowshera, and Charsadda. The images were taken at different time stamps
starting from 6H to 10H of the incubation period, which makes our dataset more generalized. Our dataset contains about 8k images with 11k instances of E. coli.

Dataset Labeling:

As MLSB media is specific to E. coli and after 24 Hours of incubation at 37°C, it results in yellow color for E. coli and pink for other types of bacteria. In the 6H to 10H, all the colonies

look transparent or very light yellow or pink which is very difficult to identify at that time whether it is E. coli or other bacteria or some salt particles. To make this easy we kept the 24H data as a reference and annotated E. coli colonies accurately and precisely in the 6-10 hours of data.

Model Training:

YOLO (You Look Only Once) is a deep learning model mostly used for real-time object detection problems in computer vision which has addressed the issues of the traditional object detection algorithm. A recent release of Yolo is version 8. YOLO network is mainly composed of three parts i.e., backbone, neck, and head which are responsible for feature extraction, feature aggregation, and generating detection respectively. For the detection of E. coli, we have trained yolov5s [22] and yolov8s [23] models.

Yolo-v5 is the fifth iteration of YOLO, in which CSPDarknet53 is used in the backbone for feature extraction, Path Aggregation Network (PANet) [24] in the neck section for successful generalization on different scale objects, and head which contains detection layers to learn to detect objects of certain sizes. In general, small objects like in our case require higher resolution features and a large number of bounding boxes. YOLO-v5 automatically updates the anchor boxes for the dataset while training. For inferencing speed and accuracy tradeoff, yolo-v5 available is available in various sizes, namely YOLOV5n, YOLOV5s, YOLOv5m, etc. These networks are only different by the number of parameters, yolov5n has the lowest number of parameters and the highest inference speed followed by s and so on. Yolov5 is trained on the COCO dataset and its various flavors performances are shown in the Figure. For the detection of E. coli, we did transfer learning and fine-tuning in yolov5s which has a good performance both in terms of accuracy and inferencing speed and achieved and mean average precision (mAP@50) of .949 shown in Figure 5.

Yolov8 is the latest release of the YOLO family. YOLOv8 is designed to be fast, accurate, and easy to use, making it an excellent choice for a wide range of object detection and tracking, instance segmentation, image classification, and pose estimation tasks. YOLOv8 has an anchor-free architecture, with an improved backbone network. Yolov8 is also available in various sizes, namely YOLOv8n, YOLOv8s, YOLOv8m, etc. For our dataset, we did transfer learning and fine-tuning in YOLOv8s and achieved mAP@50 of .950.

Mean Average Precision (mAP):

It is a commonly used metric to evaluate the overall performance of object detection models. It takes both precision (1) and recall (2) across different confidence thresholds. Average precession (3) calculates a precision-recall curve for a single class while mAP is the mean of the average precision n calculated for all the classes. In the equations, Precision, Recall, and Average Precision are represented by P, R, and AP respectively. TP shows True Positive and FP shows False Positive.

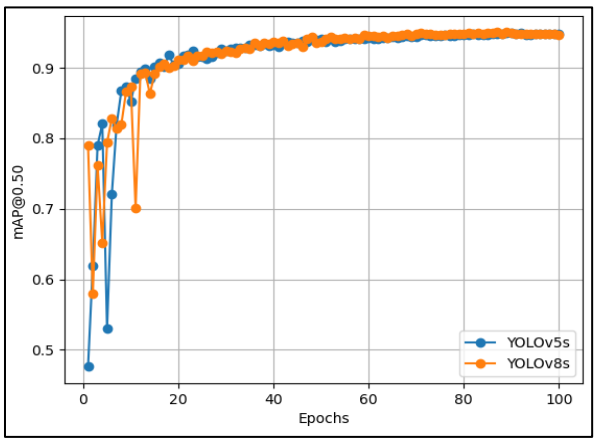

Figure 5: Comparison of YOLOv5s and YOLOv8s on current dataset

$$R = \frac{TP}{TP + FP} \quad (1)$$

$$R = \frac{TP}{TP + FN} \quad (2)$$

$$AP = \int_0^1 p(r)dr \quad (3)$$

$$mAP = \frac{1}{k}\sum_{i=1}^{k} APi \quad (4)$$

Model Deployment:

The trained model is deployed in an automatic independent kit shown in Figure 6 on Raspberry Pi. This kit has a tray for keeping the petri dish and servo motors for moving the petri dish in front of a microscopic camera for taking images which are then processed by Raspberry Pi. Also, the model was deployed on a local server for the remote users using our developed portable mobile application kit shown in Figure 7.

Figure 6: Automatic Imagery System Used for Making Dataset

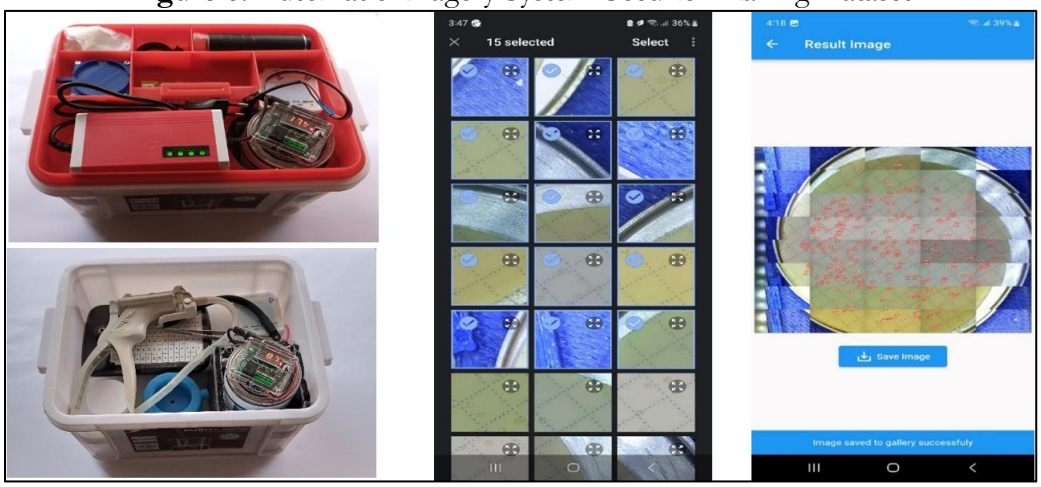

Figure 7: Manual Portable Mobile Application Kit for Remote Users

Results and Discussion:

The final goal is to test our system on the minimum possible number of CFU field samples because samples having a large number of CFUs are easy to detect after a few hours of incubation. So far, we have tested our system for more than 200 field experiments, in which we got 36 experiments having less than 20 CFUs and 27 experiments are correctly classified. In Figure 1, the blue line shows the number of CFUs detected by our system in 7H to 8H and the orange line shows the number of CFUs after 24H. Experiments having more than 20 CFUs are

all correctly classified by our system in 7H to 8H. All these testing water samples were taken from different areas in which the system shows good performance. This gap between the lines could be reduced by adding more data to our system and following the SOPs while testing and capturing images.

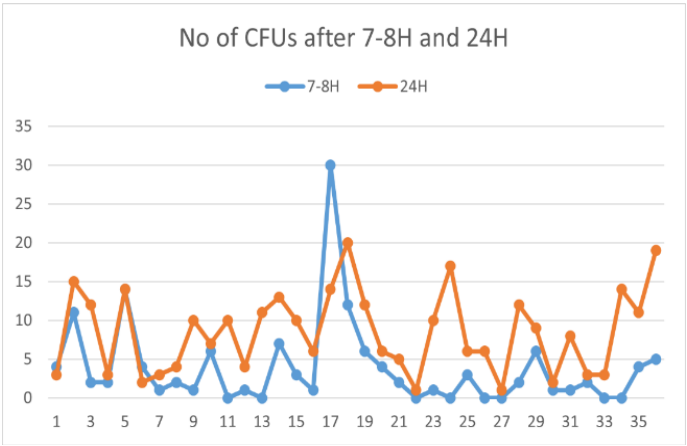

Figure 8: Performance of the Developed Systemin the field

Conclusion:

In this paper, an easy-to-use, accurate, and portable kit is presented for the early detection of up to 1 CFU of E. coli bacteria in 100ml drinking water. This system not only reduces the detection time of E. coli bacteria but also reduces power consumption. An existing accurate methodology is used which makes our system accurate and easily integrable in the existing Delagoa kit. Both the automatic and manual methods are proposed for local and remote users. The data is automatically stored on the server after processing through which the system performance improves with time.

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