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The Analysis of Staphylococcus aureus Bacteria after Exposure to Electromagnetic Radiation via Support Vector Machine

Angelica Cordero-Samortin
School of Graduate Studies, School of EECE
Mapua University
Manila, Philippines
angelsamortin26@gmail.com

Jennifer C. Dela Cruz
School of Graduate Studies, School of EECE
Mapua University
Manila, Philippines
jennycdc69@gmail.com

Ramon Garcia
School of Graduate Studies, School of EECE
Mapua University
Manila, Philippines
rggarcia747@yahoo.com

Zoren Mabunga
School of Graduate Studies, School of EECE
Mapua University
Manila, Philippines
zorenmabunga@gmail.com

***Abstract* - Microbiology is a field that is explored by many biomedical practitioners and other professionals coming from different fields. It deals with microorganisms that are too tiny to be recognized by the human eyes. Staphylococcus aureus is a microorganism considered as a major bacterial human pathogen. It is found on the skin and mucous membranes which causes a wide variety of diseases like bacteremia, infective endocarditis, skin and soft tissue infections. Several studies showed that inhibitory effect of GHz and mobile phone electromagnetic radiation to microorganisms exists. In this research, Staphylococcus aureus were exposed to electromagnetic radiation with frequency of 880MHz which is within Ultra High Frequency (UFH) range. After incubation, the images of the bacterial colonies were captured and analyzed using the algorithm in Matlab which is the Support Vector Machine (SVM). Simulation results showed that from the exposed sample, there were 1,081 colonies. From the unexposed sample, 1,190 colonies were found. These results show inhibitory effect of the electromagnetic radiation to Staphylococcus aureus. This outcome can be used in exploring other applications of electromagnetic radiation and the use of image processing in microbiology and broaden applications of machine learning.**

Keywords - Electromagnetic radiation, Staphylococcus aureus, Support Vector Machine

I. INTRODUCTION

Microbiology is a field that is explored not only by microbiologists and medical practitioners but also by those professionals coming from other fields. Microbiology is the branch of science dealing with organisms that can only be seen using devices like microscope. Microorganisms are the most ample living thing on our planet [1]. Microorganisms may be beneficial or they can be harmful too. One of them is the Staphylococcus aureus. These major bacteria can cause variety of diseases like bacteremia, infective endocarditis, skin and soft tissue infections. These bacteria which include the Methicillin Resistant Staphylococcus aureus (MRSA) originate on the skin and mucous membranes. Humanity is the major carrier for these beings [2]. One way to have a good view of these microorganisms is by digital image processing. It is the handling a picture in a digital setting with the use of a digital scheme [3]. It is done to deliver a better visual feature and simulate some mathematical procedures on pictures. More commonly, the mathematical based algorithms are used in image processing and can be achieved using Matrix Laboratory (Matlab) [4].

Researches about microbiology frequently depend on the identification colony forming units (CFU). Basically, it is completed by means of using the human eye in counting the colonies. The deliberation of bacteria in the culture can then be computed according to the hypothesis that each colony has raised from one single bacterium [5]. In relation to this, the study investigating the effect of mobile phones to microorganisms used CFUs in their results. The results showed that when S. aureus is exposed to radiation from a mobile phone during on-call mode, there is a reduction in its numbers [6]. Another study related to microorganism and image processing was the study about classifying bacteria. The simulation was conducted by means of using Machine Learning framework. The results have confirmed that images of bacteria captured from a microscope can be recognized and its genus can be identified as well [7].

Studies showed that when microorganisms are subjected to radiation coming from electromagnetic sources, microorganisms undergo inhibitory effects. The evaluation of this effect is done by counting the colonies that grew from the experimental samples and control samples. This method of counting microorganisms is an important aspect on the study of microbiology. Using the results of the mentioned studies, it can further be studied by means of exploring on a different value of frequency where in this study, 880MHz was used. The results can then be evaluated by means of counting the bacterial colonies using a digital technique.

This study focuses on evaluating the bacterial colonies exposed to electromagnetic radiation by means of image processing. The specific objectives are as follows: 1) To design a device that will radiate electromagnetic waves with a frequency of 880MHz, 2) To expose the Staphylococcus aureus bacteria to electromagnetic radiation and its effect on the bacterial growth from the reference and experimental samples, and 3) To analyze the captured images of bacterial colonies using the image processing technique known as Support Vector Machine as a faster way of counting the bacteria.

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The inhibitory effect of electromagnetic radiation has been investigated in various studies. This study can be a preliminary stage on exploring other possible applications of electromagnetic radiation such as in sanitation and food irradiation processes. The image processing technique can help microbiologists, and biomedical researchers in investigating microorganisms in a faster way of counting the bacteria. This study will also aid to further broaden the application of image processing and machine learning.

The research used the frequency 880MHz. The bacteria used in the experimentation are Staphylococcus aureus. The image was captured using iPhone4s. The captured photos contain some background noise due to the presence of unnecessary lighting. The experimentation on the exposure of the bacteria was executed only once for the reasons of financial consideration with regards to the cost of the provision of the bacteria sample and laboratory equipment.

II. Related Literature

A. Microbiology

Microbiological research techniques, according to Brugger et.al, often depend on identification of colony forming units (CFUs). This is done by allocating a small portion of a liquid culture and plating out several serial dilutions onto culture plates. These plates are Petri dishes that contain semisolid medium. After the process of incubating the samples, the colonies are then counted to define the number of CFU. The counting of colonies is done by human eye and not by a machine where plates are illuminated with light. The quantity of bacteria in the reference culture is then calculated based on assuming that each colony has grown from a single bacterium [5]. The population of microorganisms in a batch culture has different stages of growth. These include the lag phase or the stage where it gets acquainted to the new medium, the exponential phase or log phase where it grows very rapidly, the stationary phase where the microbial growth to slow down and the death phase [8].

B. Staphylococcus aureus

Staphylococcus aureus is a foremost bacterial human pathogen that creates many clinical manifestations and multiple human infections, like bacteremia, infective endocarditis, skin and soft tissue infections. These bacteria are gram-positive and are cocci-shaped and tend to be arranged in clusters that are described as grape-like [9]. S. aureus cells appear perfectly spherical with a diameter of 0.5 to 1 μm [10].

C. Effect of 2.45 GHz to L. rhamnosus

Microorganisms can be affected by electromagnetic radiation. One study used 2.45GHz was used and exposed it to L. rhamnosus. The bacterial culture used 15ml of sterile conditions. During the test, there were 8 control groups and test groups. The antenna used was 2.45 GHz dipole antenna with 1 watt maximum power density tubes. The groups were one-hour intervals for 8 hours. Based on the results, the experimental group grows less than the control group. As a result, the electromagnetic radiation has affected the bacteria of L. rhamnosus [11].

C. Effect of Mobile Phone Radiation to S. aureus

Mobile phone electromagnetic radiation has an effect on Staphylococcus aureus. This is according to a preliminary experiment investigating on its effect on bacteria that exist in the ears. S. aureus is a normal microorganism that can be found in the external auditory canal (EAC). The tests are done using the LG KG 285 model with GSM 900MHz, and peak power of 0.1 to 2 watts. The growth was investigated. The duration of exposing the bacteria was varied. The amount of growth was then checked. The S. aureus growth was hindered when it received radiation from mobile phone [12].

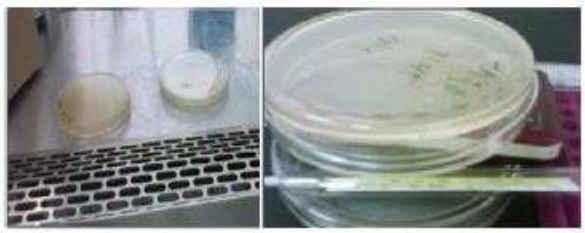

Fig. 1. Experimental setup using GSM Mobile Phone

D. Use of Electromagnetic Radiation to Eliminate S. aureus

Staphylococcus aureus was the bacteria used in the study of Samortin et.al where electromagnetic radiation was used and its inhibitory effect was evaluated. The experimentation results showed that percentage of bacteria were killed after the exposure. When 1 Gigahertz was used, the 62% of the bacteria were eliminated [13].

E. Image Processing of Captured Images

Image processing is an emerging technology used in the analysis of captured images. Image processing was used in the study about classifying bacteria and Machine Learning was used. The images of the bacteria captured from microscope. To recognize the samples, programming by Python and the Keras API with Tensor Flow Machine Learning framework were used. The experimentations have established that processed images from microscope were effectively used to determine the type of bacterium [14].

F. Use of Support Vector Machine (SVM) in Image Processing

Support vector machine (SVM) is generally based on the morphological characteristics of samples. E. coli was used as the subject in a research. The experimentation was done by spreading a volume of suspension of bacterial sample on a glass slide and allow it to dry. The image acquisition and recognition system is consist of microscope, camera, image acquisition device, computer, and personally-developed software for analyzing captured photos. Converting image to black and white or binarizing it, filling areas that are not filled and traits based on morphology of the subject are selected to classify whether it is bacteria or not. Area, perimeter, roundness, axis and ellipticity are designated as the morphology characteristics of the entity [7].

G. *Image Processing of Sludge in Wastewater Treatment Plants*

There are other topics that used image processing. One had used Image Processing of sludge in wastewater treatment plants. Processes involved segmentation analysis for morphology and the utilization of the derived parameters to prototype the sludge volume index (SVI). SVI is the most important physical measurement employed to monitor the operation of an Activated Sludge (AS) plant. In this paper, filtering is suggested for separation of bacteria that look like filaments. Accuracy and other measures are used for the separation calculation. Regression models for different AS wastewater treatment plants, according to the suggested limitations of picture investigation were investigated. The demonstrating of SVI shows the importance of the recommended image analysis restrictions for observing AS plants [15].

H. *Electromagnetic Spectrum*

The range of electromagnetic signals encompassing all frequencies is referred to as the electromagnetic spectrum. Ultrahigh frequencies (UHFs) encompass the 300- to 3000-MHz range. Their frequency is measured in cycles per second (cps) or hertz (Hz). These oscillations may occur at a very low frequency or at an extremely high frequency [16].

I. *ANSYS Simulated Antenna*

ANSYS software is widely used in simulating concepts and designs. Celik et. al simulated their antenna design using this software. Their antenna is simulated using Ansys Simulation. The loss of their antenna is shown in Figure 7. It can be seen that at frequencies 900 MHz, 2170 MHz, 3610 MHz, 5760 MHz and 6700 MHz, the antenna resonates. In these frequencies, the return loss exceeds the -10 dB which is reasonable value for an antenna to operate proficiently [17].

III. Methodology

A. *Conceptual Framework*

Development of a good quality project follows a research context. This research begins with acquiring the Staphylococcus aureus bacteria. The preparation of the culture was supervised by a professional microbiologist from Infectious Disease Diagnostic Laboratory. The process started with designing the system that will radiate the electromagnetic waves. The bacteria are then exposed to electromagnetic radiation. The samples were incubated for 24 hours. The image of the incubated control samples and experimental samples were captured and then analyzed by means of image processing to identify the colonies present on each sample.

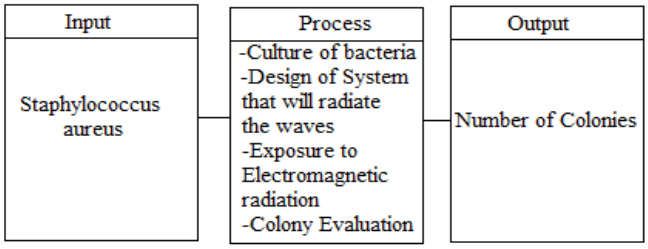

Fig. 2. Conceptual Framework

B. *System Block Diagram*

The synthesizer is wide band and it generates a range of frequencies from the single reference frequency. For this, it is 25MHz. The output frequency of the integrated oscillator ranges from 2.2x10^9 Hz to 4.4x10^9 Hz. The filter eliminates some undesired higher frequencies that might pass out of the phase detector and appear in the voltage controlled oscillator tune line. The matching circuit uses LC network for matching the output impedance of the synthesizer. The RF Pre-amplifier is for low noise RF amplification. Signal Attenuator reduces the power of a signal without distorting its waveform. Low frequency cannot be used due to amplifier. To compensate for this, the signal is attenuated. The signal will be decreased in such a way that it will not exceed the input limit. DC Filter produces pure DC from an unfiltered pulsating DC. Power amplifier amplifies the level of power of a given input signal. The power amplifier has an adaptable Gain Block that has very high open loop gain and differential inputs. Low Pass Filter which allows signals with lower frequency than that of pre- determined cutoff frequency and diminishes signals with frequencies higher than the cutoff frequency. Matching circuit in the last stage in the transmitter is used to match the out of the power amplifier and the antenna. Lastly, the antenna radiates the electromagnetic signals.

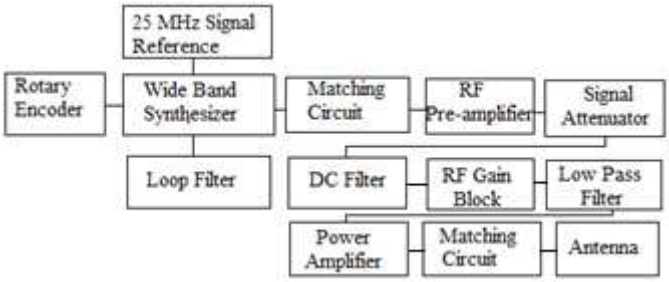

Fig. 3. System Block Diagram

C. *Antenna Design*

Bowtie is the radiating element. The simulation was done their antenna using the ANSYS software. It has center frequency of 886MHz. It effectively operates from 820 MHz to 964MHz with the return loss of -23.02 dB.

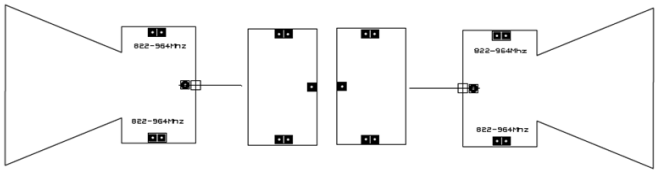

Fig. 4 Bowtie Antenna Layout

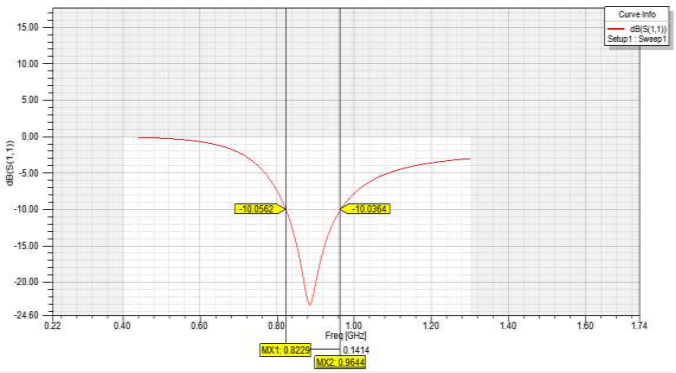

Fig. 5. Return Loss of the Bow tie Antenna

The frequency used in experimentation is 880MHz. It was used because it is within Ultra High Frequency range that was used in previous studies.

D. Experimental Procedure

Staphylococcus aureus cultures are selected for experiments. It was provided by the Infectious Disease Diagnostic Laboratory. The purity of microorganisms was secured by an American Type Culture Collection. The cultures are then processed in Tryptic Soy Broth solution and are pipetted to Petri dish with a volume of 5ml.

Fig. 6. S.aureus being processed in Trypticase Soy Broth Suspension Solution

After one hour of exposure to 880MHz, a volume of exposed and unexposed samples are streaked to Blood Agar Plate (BAP). And then, 24-hour of incubation follows. After incubation, the images of incubated samples were captured via iPhone4s and the numbers of colonies in each plate are evaluated.

Fig. 7. S. aureus being streaked on Blood Agar Plate

E. Exposure System

The exposure system is shown in Figure 8. It is consist of signal generator that generates 880MHz. The antenna radiates the electromagnetic waves. The Petri Dish contains the bacterial sample.

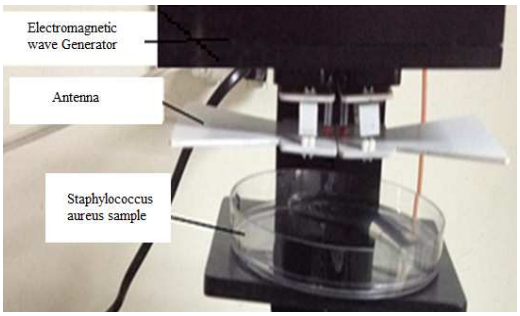

Fig. 8. Exposure system

F. Extraction of Feature Information from Images of Bacterial Colonies

Support Vector Machine (SVM)

This method is commonly used in a recognition file with multiple targets. They are used basing on the morphological characteristics of samples [7]. For this study, the characteristics used were the area and eccentricity of the colony. The images of the incubated samples were captured using iPhone 4s. The images had some noise due to unnecessary lighting. The following were done to get optimum quality of the images.

Image Processing by Color

The images were processed by means of simulating the Red Green Blue (RGB) Color Spacing of the original image.

Threshold of the Layers

It is done by manipulating the thresholds of each RGB to achieve a lesser noise environment in preparation of converting the image to its black and white counterpart.

Binarization

It is the technique where an image is consists of black and white shades.

Structuring Element

Structuring element is a morphological operator wherein the shape used is a disk and its shape is adjusted using its radius. The disk resembles the shape of the Staphylococcus aureus which has a grape-like appearance.

Extract Features

To extract features such as the area and eccentricities, the region properties were examined. Using these features, the number of colonies was quantified.

V. Results and Discussion

The experimentation involved two samples: the reference or unexposed sample and the experimental or the exposed sample. Figure 9 shows the image of colonies from the unexposed sample after the 24- hour incubation. The image was captured using iPhone 4s.

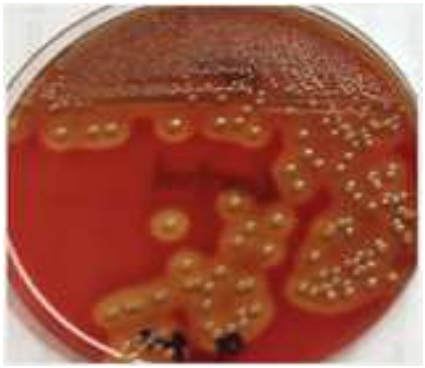

Fig. 9. Colonies from Unexposed sample

Using Matlab, the unexposed image was analyzed by simulating the threshold of the colors. The binarized image is shown in Fig. 10.

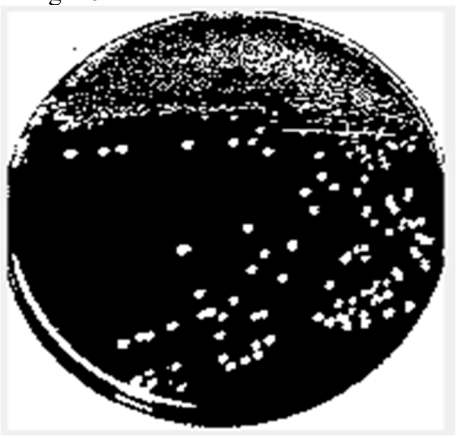

Fig. 10. Binarized Images of S. aureus Colonies from Unexposed sample

Support vector machine or SVM generally uses the morphological characteristics of a subject [15]. The morphology characteristics considered in this study are area and eccentricity. Features extracted from the simulation are shown below.

TABLE I. EXTRACTED FEATURES OF SOME COLONIES FROM UNEXPOSED SAMPLE

Colony no.	Area	Eccentricity
1	34	0.868406718
2	28	0.968050238
3	32	0.956680327
4	6	0.833333333
5	8	0.547722558

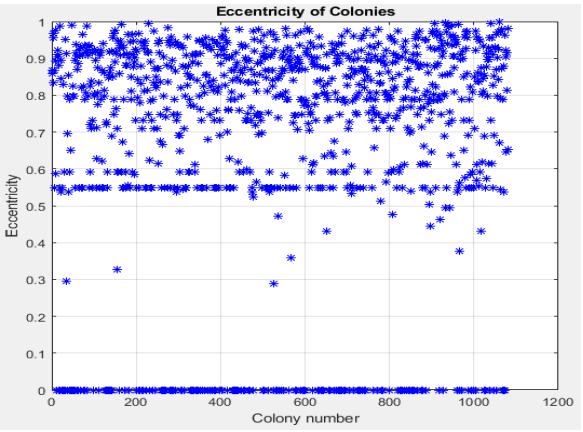

Fig. 11. Eccentricities of Colonies in Unexposed Sample

Eccentricity value ranges from 0 to 1. Eccentricity of 0 means it is circle. 1 means line segment. From the data, eccentricities were from 0 to 0.999152373. All these were counted. There were 1,190 colonies found on the sample.

Fig. 12. Exposed sample after incubation

Fig. 12 shows the image of exposed sample after the 24-hour incubation. After color spacing and color thresholding, the image undertook binarization technique. The image of colonies from the exposed sample after binarization is shown in Fig. 13.

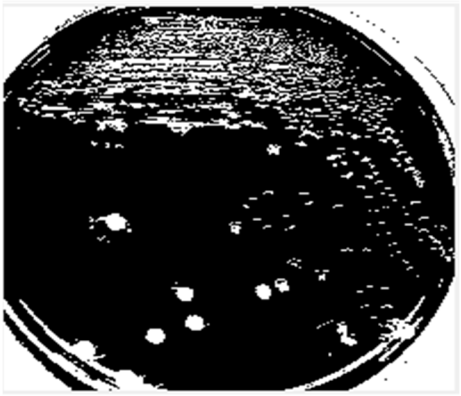

Fig. 13. Binarized Images of S. aureus Colonies from Exposed sample

Table II shows the features of some of the colonies extracted from the Matlab simulation. Based on simulation, there were 1,081 colonies found in the exposed sample.

TABLE II. EXTRACTED FEATURES OF EXPOSED SAMPLE

Colony no.	Area	Eccentricity
1	26646	0.846437335
2	8	0.894059341
3	22181	0.971449679
4	18420	0.837218411
5	35	0.943414402

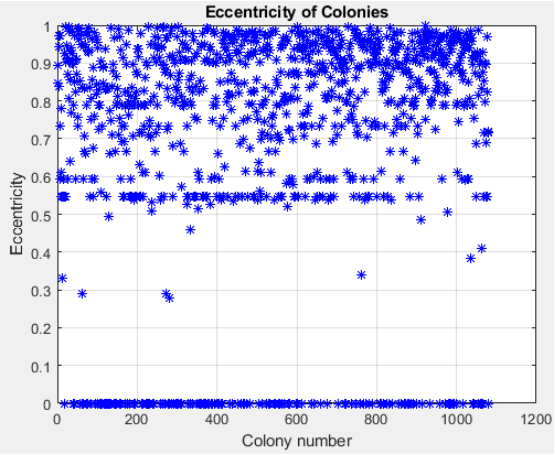

Fig. 14. Eccentricities of Colonies in Exposed Sample

III. CONCLUSION

This research deals with exposure of Staphylococcus aureus to electromagnetic radiation. A device was designed to radiate electromagnetic waves with a frequency of 880MHz. After the exposure of the bacteria to radiation, the samples were incubated. The images of bacterial colonies from the exposed and unexposed samples were captured and evaluated using image processing technique known as Support Vector Machine with the use of the software, Matlab. The images were analyzed to determine the number of colonies in each sample. Based from the simulation, the number of colonies that grew from the exposed sample is 1,081. On the other hand, there were 1,190 colonies found in the unexposed sample. This inhibitory effect can further be explored in other fields of sciences. This study can be a preliminary stage on exploring other possible applications of electromagnetic radiation such as in sanitation and food irradiation processes. The analysis approach can be broadened using other applications in image processing and machine learning.

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