EXTENDED ESSAY

RESEARCH QUESTION: What is the effect of frequency of usage in addition to temperature on the number of *Staphylococcus epidermidis* colonies in which the samples were taken from a desk by the windowsill, a desk in the middle of the classroom and a desk near the door that are examined in the medical laboratory?

SCHOOL NAME: TED ANKARA COLLEGE

CANDIDATE'S NAME: Asya BAVBEK

CANDIDATE'S NUMBER: 001129-0093

SUPERVISOR NAME: Gonca ESENDEMİR

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ABSTRACT

The experiment evaluates the effect of changes in indoor environment on the number of colonies of *Staphylococcus epidermidis*. *Staphylococcus epidermidis* is found in human skin, and it can result in serious illnesses. The research question is **'What is the effect of frequency of usage in addition to temperature on the number of *Staphylococcus epidermidis* colonies in which the samples were taken from a desk by the windowsill, a desk in the middle of the classroom and a desk near the door that are examined in the medical laboratory?'**

The samples were taken from the windowsill desk, desk in the middle of the classroom and desk near the window to observe changes in the number of colonies according to the frequency of usage and temperature. The number of colonies was counted in the laboratory by using sample cultivation, gram staining and differentiation. Five trials were conducted and the mean values were calculated to increase the accuracy of the results.

The results of the experiment illustrated that the highest mean value of the *Staphylococcus epidermidis* colonies is found in the desk in the middle of the classroom, which has the highest temperature and also people come into most contact with that desk. The lowest mean value of the *Staphylococcus epidermidis* is found in the desk by the windowsill, which has the lowest temperature and people come into least contact with it.

Word Count: 229

1.INTRODUCTION

I read the article 'Occurrence of Bacteria and Viruses on Elementary Classroom Surfaces and the Potential Role of Classroom Hygiene in the Spread of Infectious Diseases' from the website 'The Journal of School Nursing' . The article was about an experiment done in different classroom environments; therefore I wanted to conduct a similar experiment to discover the distribution of bacteria within my classroom.

Bacteria constitute a large domain or kingdom of prokaryotic microorganisms. Bacteria are found to be the causes of diseases and some certain ones are spread through surfaces: *Staphylococcus*, *Escherichia coli*, *Enterococcus* and *Salmonella*. Staphylococcus toxins are the main effect of food poisoning, and kidney failure is caused by *Escherichia coli*.[1]Important clinical infections caused by *Enterococcus* include urinary tract infections, bacteremia, bacterial endocarditis, diverticulitis, and meningitis. *Salmonella* can cause typhoid, paratyphoid and food poisoning.[2] NSF International (NSF), an independent, not-for-profit organization, recently collected and tested samples and found as many as 2.7 million bacterial cells per square inch on common school surfaces such as water fountains, desks, computer keyboards, bus seats and cafeteria trays. In the research it is found that, commonly cleaned areas, such as desks and doorknobs had 19 bacterial cells per square inch and 5 bacterial cells per square inch respectively, while computer keyboards and ear phones had significantly

more at 260 bacterial cells per square inch and 740 bacterial cells per square inch respectively.[3]

In addition to abiotic factors, the reason there is a difference between the numbers of bacteria is that even though desks, doorknobs, computer keyboards and earphones are all used frequently, doorknobs and desks are cleaned more than keyboard and earphones. There are number of factors that affect the growth and production of bacteria: Temperature, humidity and oxygen concentration and in this experiment: frequency of usage. As stated before, even though lots of bacteria such as *Staphylococcus, Escherichia coli, Enterococcus* and *Salmonella* are likely to be found in a classroom environment, *Staphylococcus epidermidis* is chosen to be the one to be experimented on since the environment in the classroom is most suitable for the growth of this particular bacteria.

Staphylococcus epidermidis is a gram-positive bacteria, and one of over 40 species belonging to the genus *Staphylococcus*. It is part of the normal human flora, typically the skin flora, and less commonly the mucosal flora. Although *Staphylococcus epidermidis* is not usually pathogenic, patients with compromised immune systems are at risk of developing infection.[4]

1: http://en.wikipedia.org/wiki/Bacteria

2: http://en.wikipedia.org/wiki/Enterococcus,

3: http://www.scrubclub.org/info/release090405.aspx

4: http://en.wikipedia.org/wiki/Staphylococcus_epidermidis

Optimal growth occurs between 30-40 °C. Furthermore the presence of oxygen is essential for the growth. Moreover NaCl level is crucial and optimal growth occurs when there is 10% NaCl.[5] According to these growth conditions and due to the fact that *Staphylococcus epidermidis* is found in human flora, the probability of finding colonies in the classroom environment is high.

Since *Staphylococcus epidermidis* is affected by temperature and frequency of usage of the area experimented on, it is logical to expect a difference of this bacteria within classroom in locations such as a desk by the windowsill, a desk in the middle of the classroom and a desk near the door. Windowsill desk could be stated as the one that is in the area that has the lowest temperature. Due to observations during the day the samples were taken, it can be stated that eleven people came into contact with the desk by the windowsill; thirty-three people came into contact with the desk in the middle of the classroom, and twenty-one people came into contact with the desk near the door. Since abiotic factors and frequency of usage differs between desks, it is logical to expect a difference in the numbers of *Staphylococcus epidermidis* within classroom.

5: http://en.wikipedia.org/wiki/Staphylococcus

My research question is 'What is the effect of frequency of usage in addition to temperature on the number of *Staphylococcus epidermidis* colonies in which the samples were taken from a desk by the windowsill, a desk in the middle of the classroom and a desk near the door that are examined in the medical laboratory?' The experiment is conducted in a laboratory where a sterilized environment is provided to ensure better results.

'Occurrence of Bacteria and Viruses on Elementary Classroom Surfaces and the Potential Role of Classroom Hygiene in the Spread of Infectious Diseases' is an experiment that was conducted in order to discover the bacteria and viruses in classroom surfaces. The presence of microorganisms on common classroom contact surfaces (fomites) was determined to identify the areas most likely to become contaminated. Six elementary school classrooms were divided into control and intervention groups (cleaned daily with a quaternary ammonium wipe) and tested for heterotrophic bacteria. Three classrooms were also tested for norovirus and influenza A virus. Frequently used fomites were the most contaminated; water fountain toggles, pencil sharpeners, keyboards, and faucet handles were the most bacterially contaminated; desktops, faucet handles, and paper towel dispensers were the most contaminated with viruses.'[6]

6: http://www.waksman-foundation.org/labs/rochester/dilution.htm

After reading that experiment, I decided to conduct an experiment to determine the bacteria in my classroom. Furthermore, I realized that the most frequently used items had the highest number of bacteria and viruses. This conclusion led me to find the independent variables, which are determined to be different locations that are a windowsill desk, a desk in the middle of the classroom and a desk near the door. According to the results of the mentioned experiment, my hypothesis is that the highest number of *Staphylococcus epidermidis* colonies are found in the middle of the room because the highest temperature is in the middle of the classroom and that specific desk is the one that is used most frequently; the lowest number of *Staphylococcus epidermidis* colonies are found in the windowsill desk because the windowsill desk is the least frequently used, and the part of the classroom which is by the windowsill has the lowest temperature. Both temperature and frequency of usage are used to detect the differences between the numbers of colonies at locations because they are highly related in this experiment in a way that if a location has the highest temperature, that same location has the highest frequency of usage. Similarly, if a location has the lowest temperature, that same location has the lowest frequency of usage.

2. METHOD DEVELOPMENT

The aim of the experiment is to explore the number of colonies of *Staphylococcus epidermidis* in an average classroom since different locations have different environmental

conditions in addition to frequency of usage. *Staphylococcus epidermidis* is chosen because it is the bacteria that have the highest probability of existence in a classroom due to favorable conditions for its growth such as temperature and frequency of usage of the surfaces.

Therefore my research question is 'What is the effect of frequency of usage in addition to temperature on the number of *Staphylococcus epidermidis* colonies in which the samples were taken from a desk by the windowsill, a desk in the middle of the classroom and a desk near the door that are examined in the medical laboratory?'

The dependent variable is the number of *Staphylococcus epidermidis* colonies because the aim of this experiment is to find the number of bacteria in different locations, which are the desk by the windowsill, a desk in the middle of the classroom and a desk near the door in a classroom. These different locations differ mainly by frequency of their usage and temperature, which was assumed to cause a difference in number of *Staphylococcus epidermidis.*

Thus, the independent variable is different locations in a classroom, which the sample was taken that are a windowsill desk, a desk in the middle of the classroom and a desk near the door. The independent variables are chosen this way because a windowsill desk, a desk in the

middle of the classroom and a desk near the door are the locations that have the most significant differences between the temperature and frequency of usage.

There are several controlled variables. First, the same kind of sterile cotton stick is used to take the samples from the different surfaces. Another controlled variable is the exact time the samples are taken. All the samples are taken at the end of the school day due to the fact that the surfaces can be considered relatively clean in the beginning of the day. To ensure better results of the quantity of *Staphylococcus epidermidis,* the samples are taken at the end of the same day. Additionally, five trials were conducted for each different location to ensure more precise results.

There are several ways of counting the number of bacteria:

1) The Serial Dilution

This is a usual method to spread bacteria over a wide range of area. Each bacterial cell in the first sample produces only one colony, if the bacteria are spread efficiently. Generally, bacterial samples must be diluted to get rational results.

2) Membrane Method

0.1 L sample is distributed past a nearly 0.5 cm membrane using different equipment. All the organisms in the sample are gathered on the membrane. Then, a nutrient medium is used for filter to be set up in. That nutrient medium helps organisms to produce and grow in size. Some separate colonies form on the surface of the membrane.

3) Using A Compound Light Microscope

The samples are cultivated in the EMB agar and Blood agar in petri dishes; they are put in an incubation device and waited for 48 hours. The colonies of *Enterococcus and Staphylococcus* are observed and then crystal violet is used to color the colonies and then iodine solution is used. After that, the slide is washed off with distilled water. Then ethyl alcohol is added and it is washed off with distilled water. Then safranin is stained and it is washed off again. Immersion oil is stained and the results are observed with the light microscope. Then catalase and coagulase are used for differentiation between *Enterococcus and Staphylococcus*.

In the experiment, the method chosen was 'Using a Compound Light Microscope' because the most accurate results are predicted to be provided in that method. Also, due to the fact that the chosen bacteria were *Enterococcus and Staphylococcus epidermidis,* differentiation

between them was more applicable in that method. Methods of sample cultivation, gram staining and differentiation are used in the experiment to obtain the desired results.

There are also some controlled variables in the laboratory. The temperature the samples are waited in the incubation device is important. All of the samples are waited at 37 °C which is the most ideal temperature for bacteria's reproduction. Also because *Staphylococcus epidermidis* are found in human flora, it can be said that they have the highest production rate at 37 °C which is nearly the same as the body temperature. The time the samples are waited in the incubation device is also essential. All of the samples are waited for 24 hours in the incubation device in order to obtain the best results of the number of bacteria and 24 hours is considered to be the optimum time. EMB agar in petri dishes is also a controlled variable because EMB agar is used to provide a cultivation area for the samples. Moreover, by using the same EMB agar, the growth conditions provided were the same for all samples. 0.05 mL of crystal violet is used in all trials to differentiate gram-positive bacteria from gram-negative bacteria and waited for 60 seconds. 0.05 mL of iodine solution is added to bind with crystal violet and waited for 60 seconds in all trials. 0.05 mL of ethyl alcohol is added for decolorization and waited for 30 seconds in all trials. 0.05 mL of safranin is stained to give decolorized gram-negative bacteria color and waited for 40 seconds in all trials. The exact waiting times for all processes are kept constant to make sure the effect is both the same and

appropriate for all samples. 0.05 mL of immersion oil is stained to make the colonies visible under microscope in all trials. Catalase and coagulase are used for differentiation between different types of bacteria to define *Staphylococcus epidermidis.*

Moreover, the pressure the samples are waited in the incubation device is another controlled variable. All of the samples are waited in the same room, thus it can be said that the pressure is constant. The pressure is measured to be room pressure: 1067.0 ±0.2 hPa. Although the pressure is usually not as important as temperature for bacteria, it should be kept the same to obtain accurate results. Furthermore, the light intensity the samples receive in the incubation device is kept the same. All of the samples are put two centimeters apart from each other under a fluorescent lamb, which provides the same light intensity for each of the samples. Light intensity is constant because higher light intensity may cause higher temperature levels. Due to the fact that temperature is a crucial controlled variable, light intensity must be kept constant to prevent any change in temperature.

2.1 Material List

- 15 sterile cotton sticks
- Light microscope
- 15 petri dishes with medium

- Incubation device
- Serum physiologic (50.0 ml ± 0.1)
- Crystal violet (0.05 ml ± 0.01)
- Iodine solution (0.05 ml ± 0.01)
- Immersion oil (0.05 ml ± 0.01)
- Safranin (0.05 ml ± 0.01)
- Ethyl alcohol (0.05 ml ± 0.01)
- Thermometer (± 0.1 °C)

2.2 Procedure:

Sample cultivation

1) 15 sterile cotton sticks are moistened with serum physiologic.

2) 5 samples are taken from the windowsill desk, the desk in the middle of the classroom and the desk near the door with cotton sticks.

3) All samples are cultivated in the EMB agar in petri dishes.

4) These samples are put in an incubation device and waited there for 24 hours.

5) After 48 hours the samples in petri dishes are observed with naked eye.

Gram staining

6) The sample is put on a microscope slide.

7) Sample is stained with 0.05 ml crystal violet 0.05 ml to differentiate gram-positive bacteria from gram-negative bacteria and waited for 60 seconds.

8) 0.05 ml iodine solution is added to bind with crystal violet and waited for 60 seconds.

9) It is washed off with distilled water.

10) 0.05 ml ethyl alcohol is added for decolorization and waited for 30 seconds.

11) It is washed off with distilled water.

12) 0.05 ml safranin is stained to give decolorized gram-negative bacteria color and waited for 40 seconds.

13) It is washed off with distilled water.

14) 0.05 ml immersion oil is stained to make the colonies visible under microscope

15) The results are observed with the light microscope.

Differantiation

16) Catalase and coagulase are used for differentiation between different types of bacteria to discriminate *Staphylococcus epidermidis* from *Enterococcus*.

3. RESULTS

3.1 Raw Data Table

Desk Location	Trials	Type of bacteria	Number of people came into contact with the surface	Temperature of the location(± 0.1 °C)	Type of Agar Plate Used	Room pressure (± 0.2 hPa)	Temperature of the incubation device(± 0.1 °C)	Radius of Agar Plate (cm)(± 0.1)	Time for petri dishes in incubation device (hour)	Number of colonies foun
Windows	Trial 1	*S.epidermidis*	11	20.0	EMB	1067.0	37.0	6.0	24.0	20 colonies
	Trial 2	*S.epidermidis*	11	20.0	EMB	1067.0	37.0	6.0	24.0	23 colonies
	Trial 3	*S.epidermidis*	11	20.0	EMB	1067.0	37.0	6.0	24.0	24 colonies
	Trial 4	*S.epidermidis*	11	20.0	EMB	1067.0	37.0	6.0	24.0	24 colonies
	Trial 5	*S.epidermidis*	11	20.0	EMB	1067.0	37.0	6.0	24.0	22 colonies
Middle	Trial 1	*S.epidermidis*	33	25.0	EMB	1067.0	37.0	6.0	24.0	58 colonies
	Trial 2	*S.epidermidis*	33	25.0	EMB	1067.0	37.0	6.0	24.0	60 colonies
	Trial 3	*S.epidermidis*	33	25.0	EMB	1067.0	37.0	6.0	24.0	62 colonies
	Trial 4	*S.epidermidis*	33	25.0	EMB	1067.0	37.0	6.0	24.0	64 colonies
	Trial 5	*S.epidermidis*	33	25.0	EMB	1067.0	37.0	6.0	24.0	63 colonies
Door	Trial 1	*S.epidermidis*	21	22.0	EMB	1067.0	37.0	6.0	24.0	34 colonies
	Trial 2	*S.epidermidis*	21	22.0	EMB	1067.0	37.0	6.0	24.0	35 colonies
	Trial 3	*S.epidermidis*	21	22.0	EMB	1067.0	37.0	6.0	24.0	34 colonies
	Trial 4	*S.epidermidis*	21	22.0	EMB	1067.0	37.0	6.0	24.0	34 colonies
	Trial 5	*S.epidermidis*	21	22.0	EMB	1067.0	37.0	6.0	24.0	37 colonies

Table 1: The raw data table consists of number of colonies of *Staphylococcus epidermidis* in different temperatures, in which the samples are waited in EMB agar petri dishes, which have six centimeters radius in the incubation device for 24 hours in 37 °C at 1067 hPa.

3.2 Data Process Table

Example of calculations of number of colonies of windowsill:

Mean: (20+23+24+24+22)/5 =22.6

Standard Deviation:

* $s = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \overline{x})^2}$ =1.6773

Standard Error: Standard deviation/√number of elements

=1.6733/√5

= 0.750

Variance: (standard deviation)^2 = (1.6773)^2

= 2.8

Desk's location	Temperature(± 0.1 °C)	Mean	Mode	Median	StandardDeviation	Variance	Standard Error
Windowsill	20	22.6	24	23	1.6733	2.8	0.7483
Middle	22	61.4	-	62	2.4083	5.8	1.0770
Door	25	34.8	34	34	1.3038	1.7	0.5830

Table 2: This is the table that shows the mean, median, mode, standard deviation**,** variance, standard error of number of colonies *Staphylococcus epidermidis* found in different locations.

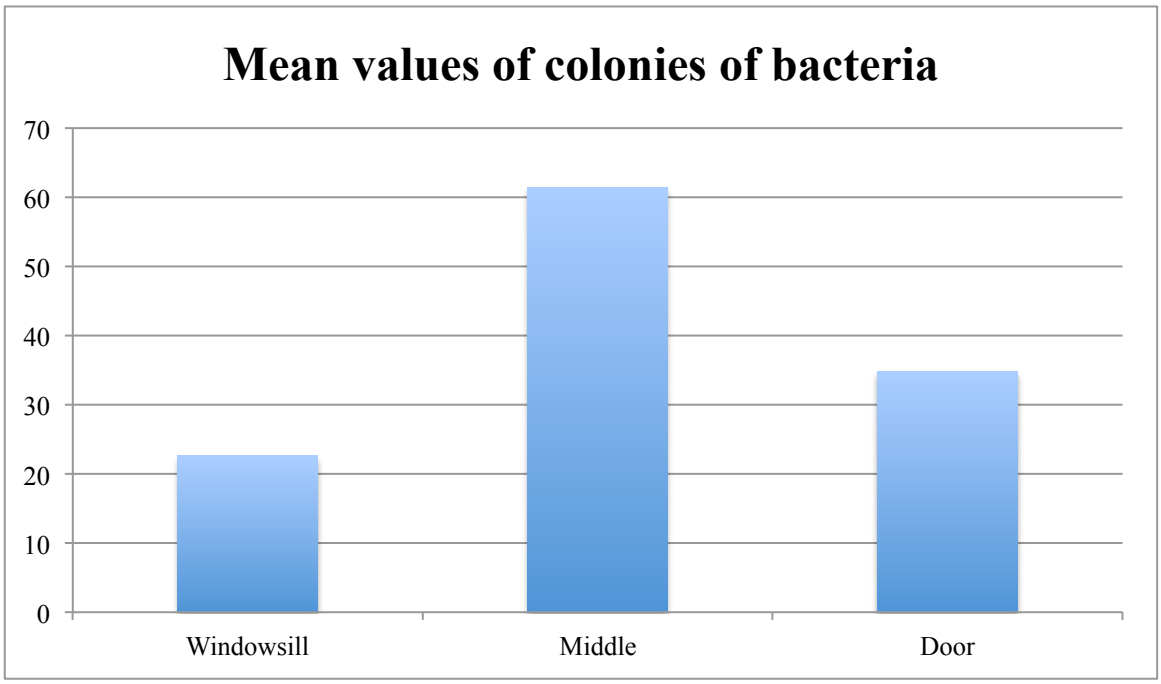
* http://www.miniwebtool.com/standard-error-calculator/

3.3 ANOVA TABLE

Location of the desk	**Trial number**	**Mean**	**St. deviation**	**SS**	**df**	**MS**	**F**	**P values**
Window sill	5	22.6	1.6733	3936.4	2	1968.2	573.3	1.235×10^{-12}
Middle	5	61.4	2.4083					
Door	5	34.8	1.3038					

Table 3: This tabled depicts mean, standard deviation, sum of squares, degree of freedom, mean square, F and P value of different locations.

3.4 Bar Graph



Graph 1: This bar graph depicts the mean values of colonies of *Staphylococcus epidermidis* and the locations the colonies were found.

4. ANALYSIS AND EVALUATION

In this extended essay, I conducted an experiment to observe the change in number of *Staphylococcus epidermidis* in different locations in a classroom environment such as a desk by the windowsill, a desk in the middle of the classroom and a desk by the door. Five samples from all the three surfaces, a windowsill desk, a desk in the middle of the classroom and a desk near the door, were taken in order to ensure the accuracy of the results. The samples are cultivated in the EMB agar in petri dishes; they are put in an incubation device and waited for 48 hours. The colonies of *Enterecoccus and Staphylococcus* are observed and crystal violet is used to color the colonies, and then iodine solution is used to bind with crystal violet. After that, the slide is washed off with distilled water. Then ethyl alcohol is added for decolorization and it is washed off with distilled water. Safranin is stained to give decolorized gram-negative bacteria color, and it is washed off again. Immersion oil is stained to make the colonies visible under microscope and the results are observed with the light microscope. In that stage, the only observation that can be done was to see the several colonies but the differentiation of the two bacteria types cannot be done. Then catalase and coagulase are used for differentiation between *Enterococcus and Staphylococcus epidermidis*. Because all the samples are taken at the end of the school day, the assumption that the *Staphylococcus Epidermidis* spread to surfaces by students and teachers can be made.

As it can be seen from the results, the mean value of *Staphylococcus epidermidis* colonies is highest in the middle of the classroom, which is 61.4 colonies; this result can be used to verify the hypothesis that was stated. The mean value of *Staphylococcus epidermidis* colonies is lowest in the windowsill desk, which is 22.6 colonies, and this result also agrees with the hypothesis. The mean value of the *Staphylococcus epidermidis* colonies in the desk near the door is 34.8. Also, in the windowsill desk, due to the fact that standard deviation was found 1.6733 and standard error was found 0.7483, it can be concluded that most of the data are close to the mean value and the data collected were accurate. For the desk in the middle of the room, due to the fact that standard deviation was found 2.4083 and the standard error was found 1.0770, it can be concluded that most of the data are further away from the mean value than the data taken from the windowsill. It can be seen that the lowest standard deviation is 1.3038, and the lowest standard error is 0.5880, which is found in the data taken from the desk by the door. It can be stated that the most accurate result was found in the data taken from the desk by the door. Moreover, The Analysis of Variance was calculated. ANOVA was chosen because the experiment had three mean values and ANOVA is used to analyze the means of two or more groups. According to this calculation, it was clear that the mean values of the number of colonies taken from different locations were not equal to each other. P value was found to be 1.235×10^{-12} which is less than 0.500; this means that the difference between

the mean values of three groups was statistically meaningful. It can be said that the results of ANOVA agrees with the hypothesis.

According to the information I received from the cleaning department of the school, the classrooms are cleaned with soup and water everyday after school hours and they are also cleaned two times on the weekends. Even though *Enterococcus and Staphylococcus* are not affected by cleansing agents, *Escherichia coli* and *Salmonella* are affected by cleansing agents. The fact that *Staphylococcus epidermidis* are not affected by cleansing agents explains the reason it was found in the samples that were taken from the windowsill desk, desk in the middle of the classroom and desk near the door. As it can be seen in the images (Appendix), all samples are observed independently by using the same method. In the image of the microscope, colonies can be observed. In the experiment, it can be said that environmental conditions such as temperature and frequency of usage affect the quantity of bacteria.

There were some strengths and weaknesses of the experiment. First of all, due to the fact that five trials were conducted, the accuracy was increased. Also, as it can be seen from the standard deviation and standard errors, the data taken were close to each other and to the mean value. This leads to the interpretation that the data were relatively accurate. Moreover,

the equipment used increased the accuracy of the results due to the fact that their sterility was exactly as intended. Furthermore, because most of the experiment was conducted in the laboratory, the optimum environment to obtain the most accurate results was set.

On the other hand, the different locations of the indoor environment, despite the fact that they had difference in temperature values and frequency of usage, could have had more significant differences. Rather than choosing the surfaces in the same classroom, surfaces from different parts of the school could have been chosen, such as choosing three classrooms which one of them is in part of the school that sees less sunlight than others, one in a part which sees a lot of sunlight and one in the middle of the school.

By observing the results of the experiment, it can be concluded that the optimum growth conditions for *Staphylococcus epidermidis* are temperature above 20 °C and below 40 °C and frequent usage due to the fact that the highest number of colonies were measured in the samples taken from the part of the room that has the highest temperature. Due to the fact that *Staphylococcus epidermidis* is found in skin flora, it was found in a high number of colonies after the end of the school day, which means the desks were came into contact with numerous people. The hypothesis that the highest number of colonies would be found in the samples taken from the desk in the middle of the room was verified. The results of the experiment

could be used to decrease the rate of outbreak of diseases by cleaning the places that are touched most frequently more than the others. As it can be said that the temperature is also an effect of the production of bacteria, the temperature in the room can be stabilized at below 22 °C. By cleaning the most frequently used areas, and by decreasing the temperature of the classroom, the health of the students can be ensured.

Despite the fact that the experiment had some weaknesses, it was possible to maintain a nearly optimum environment for the results to be obtained. There could be done further in-depth experiments on this subject, which can also connect the relationship between the effectiveness of cleansing agents and the number of bacteria found. Further disease rates can be decreased if that relationship is determined and the most effective cleansing agent can be determined. These further experiments may lead to more healthy studying conditions in schools. Also, other than schools, the same experiment can be conducted in work places to determine the number of bacteria and the rate of the spreading of diseases may be decreased. Moreover, an experiment more professional might also lead to findings with using different controlled variables and independent variables.

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APPENDIX

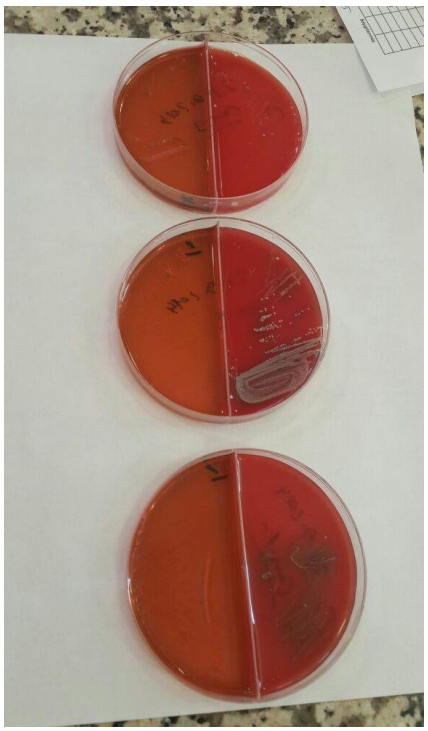

Figure 1.1: This image shows the samples in EMB Agar petri dishes taken from the desk by the windowsill.

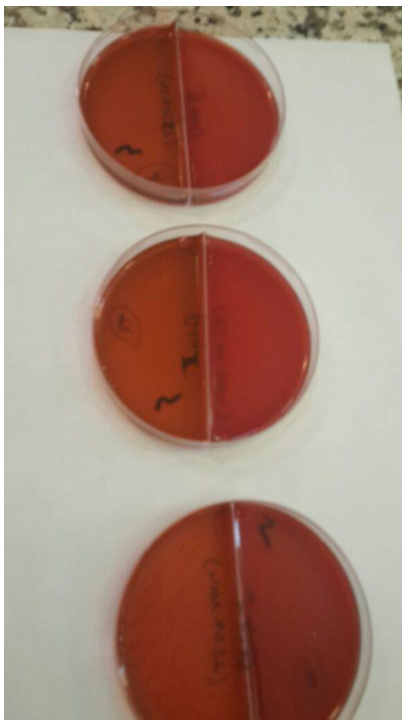

Figure 1.2: This image shows the samples in petri dishes taken from the desk in the middle of the classroom.

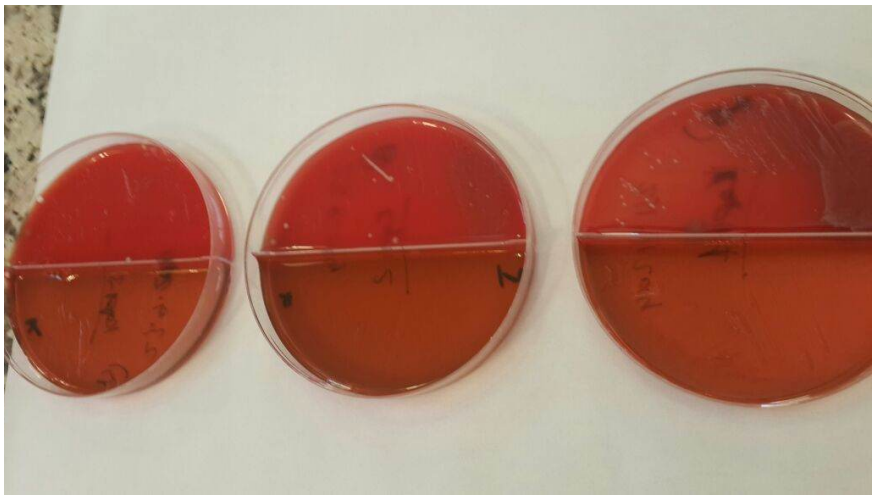

Figure 1.3: This image shows the samples in petri dishes taken from the desk near the door.

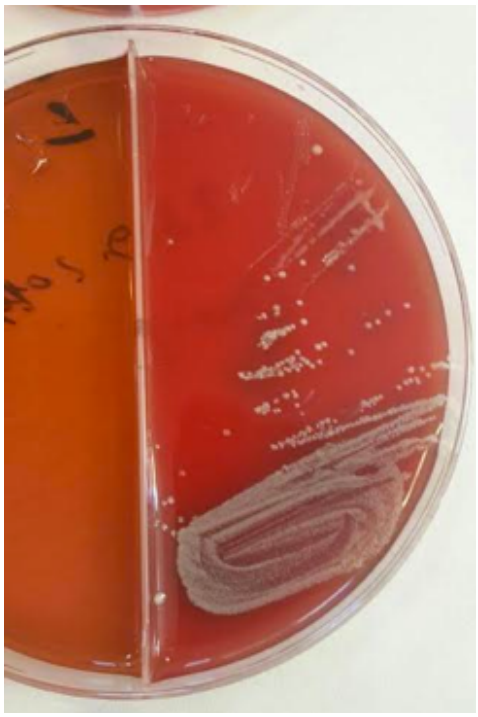

Figure 1.4: This is the image of the second trial of the sample of desk in the middle of the classroom.

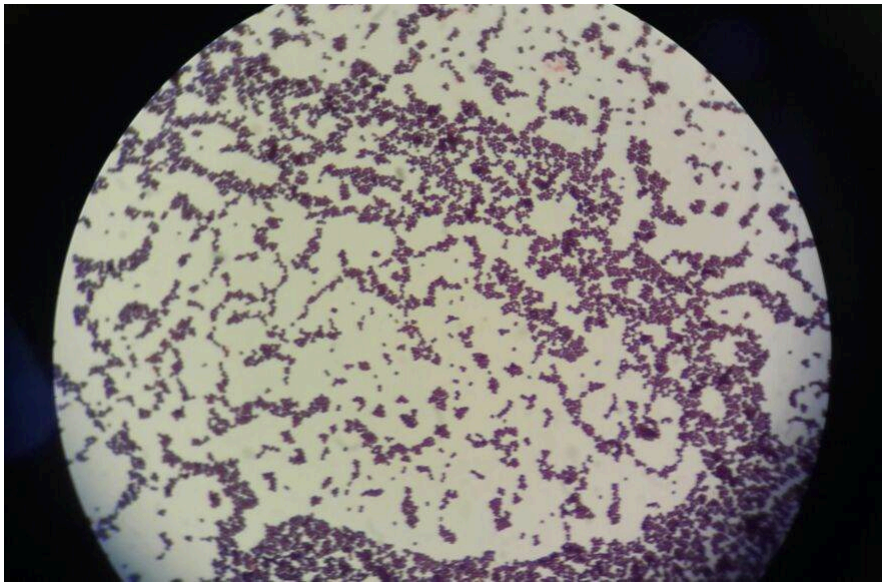

Figure 2: This is the miscroscopic image of the second trial of the sample in petri dish that was taken from the desk by windowsill desk. *Staphylococcus epidermidis* can be seen clearly.