Mesophiles and Temperature Resistant Bacteria in Urban Technology and Their Impacts on Humans

Research and Writing by: Catherine Clinton

Sponsor: Dr. William Olsen

Reader: Dr. Yan Xu

Worked Under Guidance Of: Dr. Sonya Taylor, Dr. Stephanie Holt, Dr. Thomas Owen,

Professor John Butyn

University of Glasgow, University Avenue, Glasgow G12 8QQ, UK

Ramapo College of New Jersey, Mahwah, NJ, 07430 USA

Introduction:

In the realm of microbiology, there are numerous methods available to identify and classify microbes. The varying ability for a microbe to function optimally in different pHs, or different osmotic pressures, or even temperatures, is what characterizes and distinguishes them from one another in both the natural, as well as the urban environments. Regarding temperature specifically, microbes are able to be categorized into three major groups, depending on which temperatures are more suitable for the microbe to thrive in: psychrophiles, mesophiles, and thermophiles (Turner *et al.,* 2007).

High interest in bacteria that are able to thrive in extremely high or varying temperatures rose greatly after a discovery in Yellowstone National Park. Thomas D. Brock and his colleagues discovered bacteria that were able to survive and thrive in areas of extremely high temperature (Brock, 1978). After this initial discovery, he went on to research and write various publications centered around thermophilic bacteria. Others followed as well and more and more was discovered about bacteria that could thrive at temperatures that we would consider to be quite extreme.

Bacterial temperature resistance and suitability to high temperature environments may in the future have a major impact on determining sterilization regulations, which is important for people regarding health and their well-being. In the food industry, there are usually many issues in regard to spoilage or bacterial contamination. Regarding even things such as the process of making milk powder, the temperature at which the process occurs is set between 40 and 70 degrees Celsius. This temperature range is very suitable for the growth and even count as an optimal condition for some mesophilic and thermophilic bacteria. Heat is often used to kill bacteria, but when thermophiles are resistant even up to 135°C of heat that creates a problem in getting rid of the bacteria for consumers wanting the milk (Melzoch *et al.*, 2004). Often the

thermophiles produce endospores that are highly heat-resistant, so not much is getting affected by the heat treatment done on the milk products, so widely enough the milk products are being colonized and contaminated (Melzoch *et al.*, 2004).

In order to better understand the types of bacteria that can survive at higher temperatures, processes must be done in order to identify them, classify them, see the factors influencing them, know the types of bacteria that are able to survive and thrive at higher temperatures, understand how to sequence them, as well as getting an understanding of the risks. But first, it is important to understand what a thermophile is.

Thermophiles are bacteria that grow and thrive in areas above at least 37°C, which is the average human body temperature. They live in areas of high temperature, and are often found in water (Brock *et al.,* 1972). Hot springs are major areas holding thermophilic bacteria. Geothermally heated water is in many places all around the world, and hot springs are usually highly concentrated and heated areas (Brock, 1978). Other areas in the world, where there is man-made heat, sunlight, etc., are not as prominent sources of heat for thermophiles to reside in, because there is not often as much consistency in the high temperatures (Brock 1978). Since most bacteria and microbes are usually found in less extreme environments, the thermophiles have to adapt in order to withstand such high temperatures, anywhere between 40°C and 135°C (Melzoch *et al.,* 2004).

One type of bacteria found and isolated in 1972 is known as *Thermus aquaticus* (Brock *et al.*, 1972). *Thermus aquaticus* is a thermophilic bacterial species with an optimal temperature of growth between 70°C and 75°C, and is able to survive in areas with temperatures between 45°C and 85°C (Brock *et al.*, 1972). Within this species, there was a strain founded by Ramaley and Hixson, known as X-1, which was almost the same as the discovered *Thermus aquaticus ,* but did not have the same yellow pigmentation, and also grew faster. While *Thermus aquaticus*

was found in nature in geothermally heated places like hot springs, the X-1 strain was founded in water heaters (Brock *et al.*, 1972). The Center for Disease Control Division of Bacterial Diseases researched effects of unnamed thermophilic bacteria and looked at the clinical manifestations of thermophilic infections in patients (Rabkin *et al.,* 1985). For patients, the ones infected had illnesses such as meningitis and some showed symptoms such as respiratory infection, high fever, and sepsis. Most of the time there were antibiotics that the thermophilic bacteria were susceptible to, and almost all the bacteria were susceptible to almost all antibiotics listed, with few exceptions. In at least six cases studied, the infection resulted in disease (Rabkin *et al*, 1985).

There are many factors aside from just temperature that affect the way that bacteria grow and thrive. Since often bacteria reproduce and have many generations in short amounts of time, the bacteria have evolved greatly in order to adapt to natural environmental extremes, as well as new modern extremes in the environment and bacteria's newer indoor environments. Factors that influence the way in which bacteria function are temperature, pH, and osmolarity (Adigüzel, 2009).

For most bacteria, the optimal environment to grow at is at neutral or close to neutral pH levels, which is around 7, and this is the most common pH level found naturally in most places, according to the research of Adigüzel and others. While this is true, there are also other bacteria that evolved in order to thrive in extremely different pH levels. At pH levels below 4, some acid-loving bacteria can thrive. Acidophiles are the types of bacteria that can thrive in such extreme environments. Common environments in which they can be found are in acidic environments such as some animal stomachs, or even in volcanoes. Some environments can have a higher pH as well. In instances like these the pH level would be basic. Some forms of bacteria have adapted and evolved to be able to thrive in such environments as well, and these

types of bacteria are called Alkalinophilic bacteria. These types of bacteria are able to grow optimally usually between pH levels of 9 and 10. Usually these extreme types of bacteria that grow optimally at extremely acidic or basic conditions are not able to grow at neutral or other pH levels as well, and often would die.

In regard to osmolarity, bacteria often reside in water environments (Brock, 1972). Most cells in general tend to have a high percentage of water within them. Microbes usually contain about 80-90% water inside of them. Cells often have an equilibrium in the water they have within the cell and outside of the cell, and if there is more salt in the solution outside of the cell, then the water will go towards the salt and leave the cell. This is known as plasmolysis, and occurs when the solution is hypertonic to the cells (Cummings, 2007). Because of adaptation to different environments though, and evolution, bacteria have evolved to being able to thrive in higher concentrated areas. In order to be able to survive within a salty environment, they must be salty themselves, and for these types of bacteria, the salty environment is their equilibrium. These salt lovers are known as halophiles, and reside in extremely salty environments such as the Dead Sea.

Another way in which organisms can thrive in extreme conditions is that some are more tolerant to radiation than others. Ionizing radiation resistance has been a particular topic of interest. Radiation in nature on Earth is not often occurring, but with events such as Chernobyl, or Fukushima, interest has been piqued in the presence of radiation resistance in microbes (Shuryak *et al*, 2017). These radiation resistant types of bacteria are able to be found just about anywhere these days, from sawdust, to pillows, to clinical areas.

There are many ways in which bacteria can be distinguished from one another, and can be identified through various means. Gram staining is the most common way of assisting in identification of bacterial samples *in vitro* (Beccera *et al*, 2016). The stains will either come out

as gram negative (pink) or gram positive (purple). When the resulting stain is gram negative it means that the peptidoglycan layer is thin, but if the result is gram positive the cells have a thick peptidoglycan layer (Beccera *et al*, 2016). This is commonly one of the first ways in which bacteria can be observed and differentiated.

When staining and later on reviewing under a microscope the bacterial cells should look like one of the two following images.

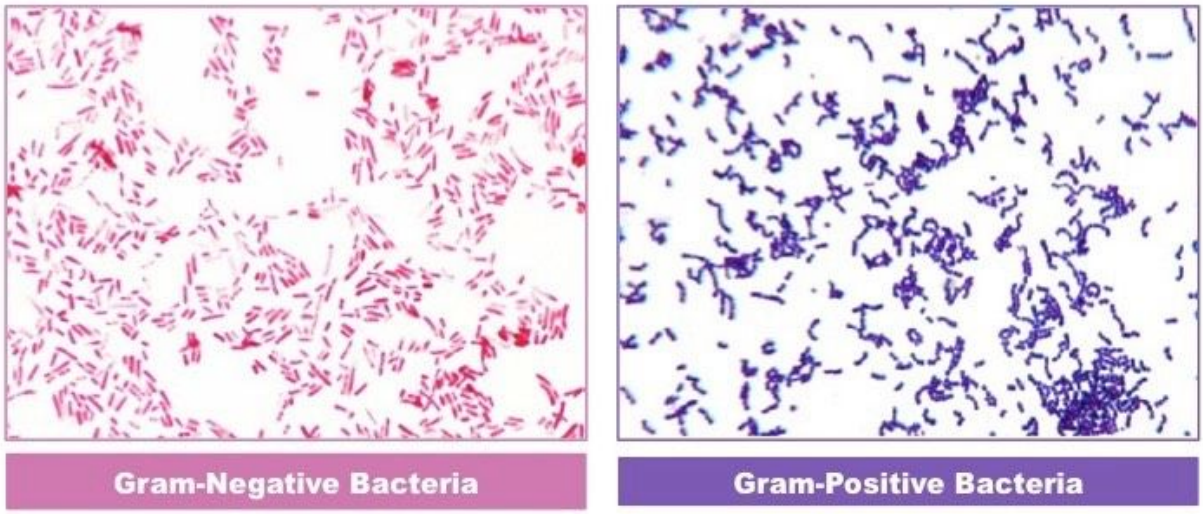


Figure 1: Differentiating between gram negative and gram positive staining under microscope

Another thing to look at is the the DNA in order to get a more exact identification. This can be done by looking at the 16s rRNA. The 16s rRNA gene in particular is chosen as the gene of choice because of its distinguishable characteristics. It is composed of 1,550 base pairs in length, making it large enough so that it has distinguishing components. It is also a gene that is universally found in all bacteria, making it easily able to compare with all other bacteria (Clarridge, 2004). After developing a culture for the bacteria, the DNA must be isolated. Once the DNA is obtained, PCR is used to amplify the 16s rRNA. 16s rRNA is commonly used in

order to identify bacteria from one another. Using 16s rRNA has been proven useful in multiple studies. Research done by Gilberto E. Flores and others on bathroom surfaces used this type of identification. Prior to sequencing, the samples were collected from various surfaces using sterile cotton swabs. The genomic DNA was extracted from the cotton swabs using MO BIO PowerSoil DNA isolation kit, while following the directions of the manufacturer along with some changes directed from another source. In this, part of the 16s rRNA was amplified using a primer set, PCR mixture conditions, and thermal cycling conditions (Flores et al., 2011).

Materials and Methods:

The methods were done in accordance with the Lab Manual *Mesophiles and Thermophiles in the Urban Environment*. The project began by taking samples from areas such as Glasgow and New York City, labelling, and bringing back to the lab for testing. The samples were taken by getting a clean sterile cotton swab in a sealable tube, dipping the swab in sterile water, then swiping the cotton swab on the selected area, testing areas that would possibly have thermophilic or mesophilic bacteria. The areas that were sampled were the lab worked in, apartments, areas in New York City such as Penn Station, restaurants, and Ramapo college. The most common things sampled were hand dryers and microwaves, but things such as stoves, faucets, and boiling racks were sampled and led to successful bacterial samples. Of the 33 different sample sources taken, 20 successful DNA samples resulted and were identified.

Once the samples are brought back to the lab, the samples are streaked onto a Nutrient Agar plate, or at Ramapo College, they were streaked onto Tryptic Soy Agar (TSA) plates. After streaking, 2ml of distilled water to the tube, the cotton swab back was put into the tube, then left in the cold room (4°C) for storage. The plates were incubated anywhere between 1 and 4 days at (37°C), and checked on to see if there was any visible growth. For the ones that had no

visible growth, the plates were thrown away and there was no further testing. If there was growth on the plate, then the process continued with isolation.

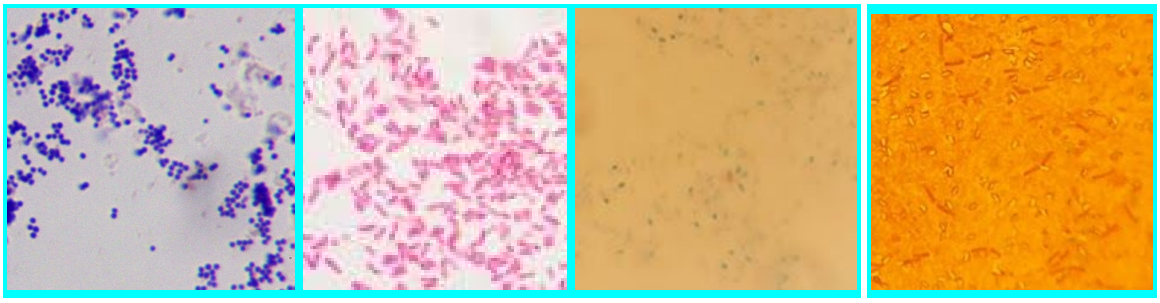
In order to isolate the colony, you must take a single colony and spread it in a small area on a nutrient agar plate using a sterile toothpick or nichrome loop. From that small patch spreaded, another toothpick is used to make 3 streaks from the small area, and another toothpick is used to make another 3 streaks from the ends of the previous streaks, and so on until that was done four times.

Figure 2: Streaking Pattern for Isolating Bacteria

When at Ramapo College, directions were given to do double isolations of the sample colonies. This was done in order to make sure that there was only the one strain of bacteria in the sample. Same directions were followed for the first isolation for each of the samples as the second isolation.

Once the isolated samples grew, various testing on each isolated bacteria began. The first testing performed was gram staining. Gram staining shows the difference in thickness for the peptidoglycan cell wall, being purple if thick and pink if thinner (Beccera *et al*, 2016). Another method of testing for identification was an endospore staining test. Endospores are

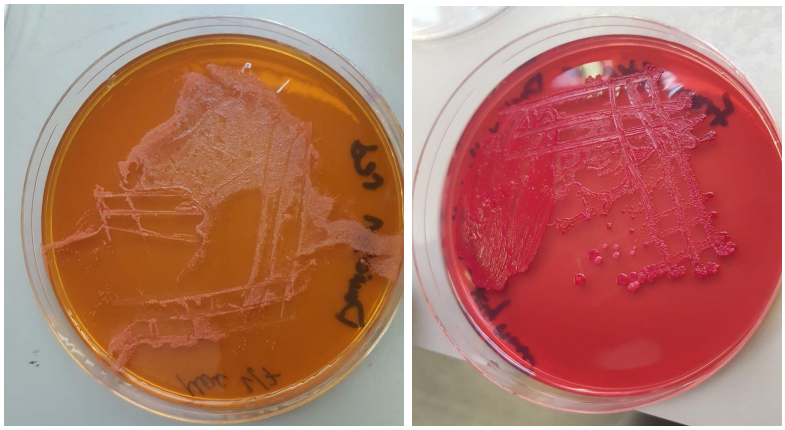
resistant asexual spores that develop inside some bacterial cells. They develop to resist death in unfavorable conditions to the bacteria, so the bacteria are able to protect themselves with the endospores. When bacteria is at a temperature that is unfavorable, endospores may form. The bacteria will have trouble reproducing, but with the endospores it survives, and is able to wait for a favorable condition to occur again to grow and thrive.



Figures 3-6: Gram Positive, Gram Negative, Endospore Positive, and Endospore Negative Stains

Other plates are also used in order to test include using selective media to further identify the samples. Four types of selective media used were MacConkey Agar, Eosin Methylene Blue agar, Mannitol Salt agar, and Sodium Azide Blood agar. The selective media is used in order to understand better the individual bacteria and see what it is capable of. MacConkey and Eosin Methylene Blue (EMB) agars are selective and work only for gram negative bacteria, and Mannitol Salt and Sodium Azide Blood agar are gram positive selective.

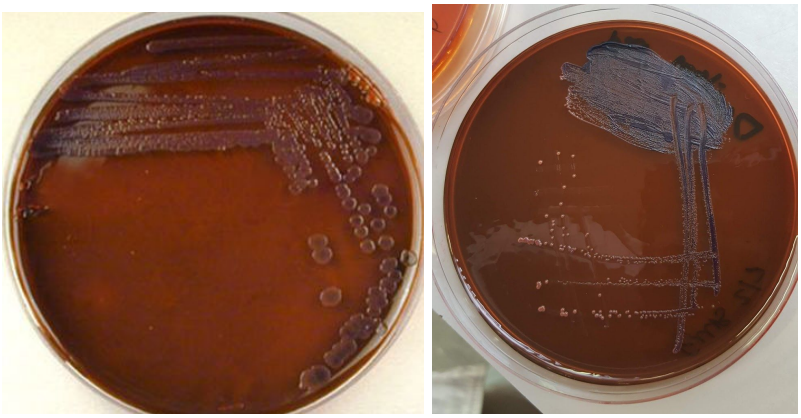
MacConkey:



Negative Positive

Figure 7 and 8: Negative and Positive MacConkey agar plates

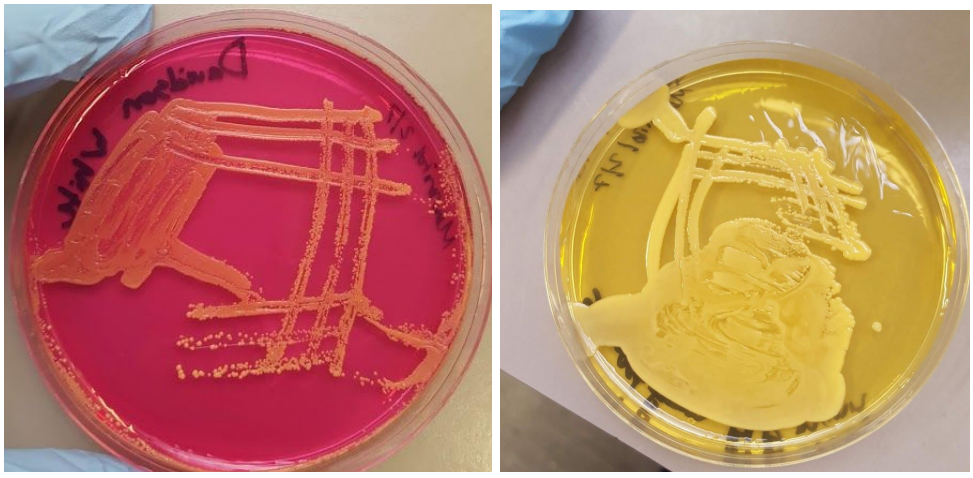
EMB:



Negative Positive

Figures 9 and 10: Positive and Negative Eoisin Methylene Blue agar plates

Mannitol Salt:



Negative Positive

Figures 11 and 12: Positive and Negative Mannitol Salt agar plates

Sodium Azide Blood:

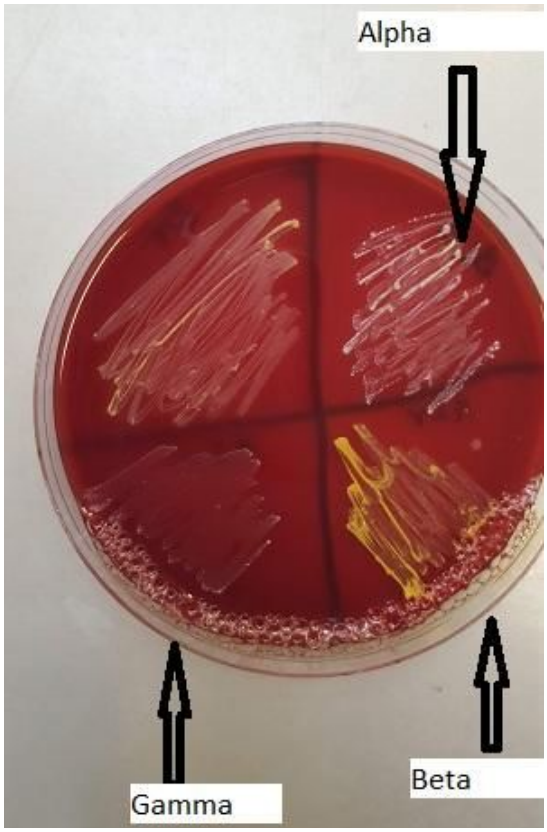


Figure 13: Sodium Azide Blood agar plates

Another type of plating used in order to get more practical information on the bacteria was antibiotic assaying. In order to do this nutrient broth was made, the broth was added to each Erlenmeyer flask, and a toothpick with one colony was added to the same flask as well. The flasks were left in the hot room (37°C) to grow overnight, and then 10µl of the broth was added to a plate. The broth was spread out evenly and then, depending on if the bacteria was gram negative or gram positive, there would be a set ring of antibiotics that would be used in order to see if any of the antibiotics were susceptible or resistant. If the bacteria is susceptible there will be a ring of clear area where bacteria was not able to grow around the antibiotic it is susceptible to, as can be seen in the figure below.

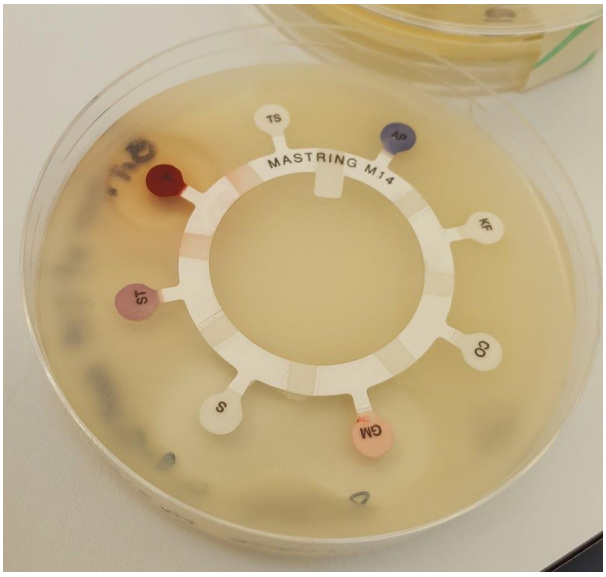


Figure 14: Antibiotic Assay showing susceptibility and resistance to bacterial sample

Once the other testings are done, identification of the bacteria found was done through processes. The process began with DNA isolation, which was done by taking a colony, mixing it with water in a microfuge tube, and boiling the sample for around 10 minutes. After this taking everything but the pellet of excess DNA was done, and the rest of the sample was put into a new sterile microfuge tube, with each tube being labelled with their respective sample names.

After this the preparations for PCR began. A master mix was made in which each of the samples were put together using the following recipe:

A typical PCR mixture (50 µl per reaction) contains:

- 10µl 5x Flexi Buffer
- 3µl 25mM $MgCl_2$
- 1µl 10mM dNTPs
- 2µl Primer 1 (8F)
- 2µl Primer 2 (519R)
- 0.25µl Taq Polymerase
- 25.75µl dH_2O
- 6µl Template DNA

Figure 15: PCR Master Mix Recipe (amount needed for one sample)

After the sample was put together, the tubes were put into the PCR machine and left to run. After PCR ran the samples were tested with gel agarose. The gel was made with 1g of agarose (in powder form), and 100ml of 1xTAE buffer, and this test was done in order to test if the sample that went through PCR was a pure sample, and if it had an appropriate amount of DNA so that it could be tested, sequenced, and identified. A ladder is added to the far left of the gel so that there is a set standard for which it is known where the samples should run up to in order to measure as the appropriate length of base pairs. The samples should all run to around the same area, since the samples that were amplified should be around 600 base pairs in length.

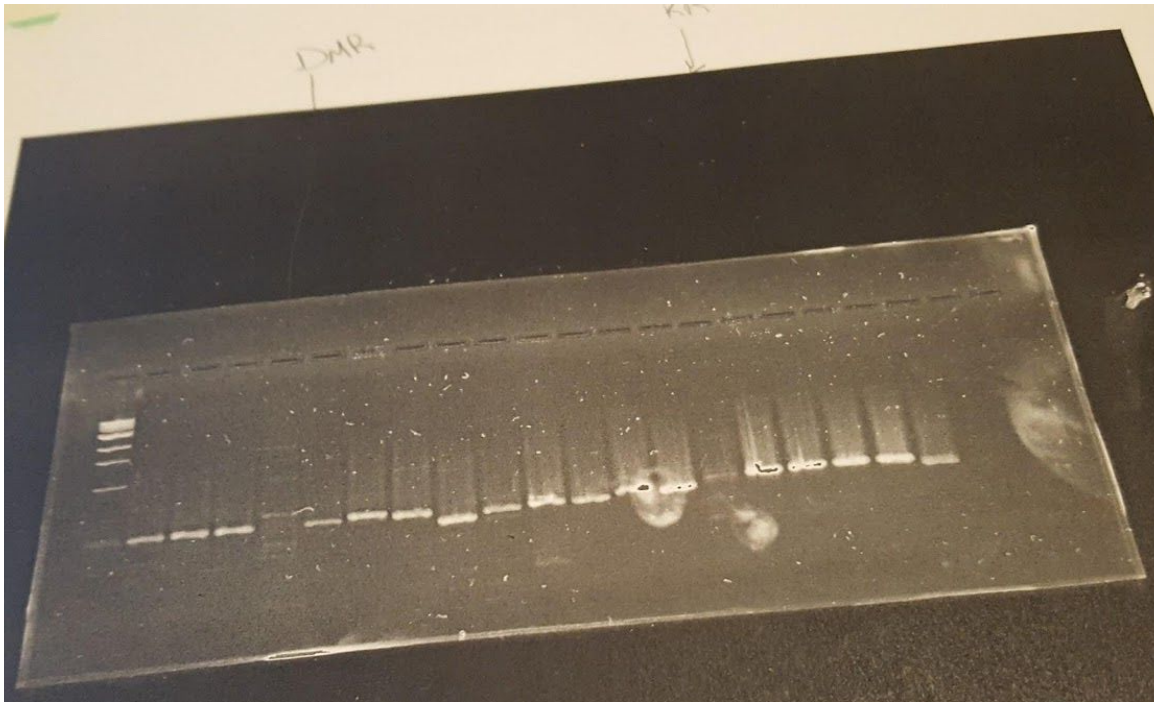


Figure 16: DNA Gel Agarose testing for 20 Bacteria DNA samples

After this ran successfully, the samples were cleaned up using the Qiagen PCR purification kit, and then ran again on a gel to check that the sample still had what was necessary for sequencing. And in order to check the concentration, a nanodrop test was used. In order to determine how much of the cleaned up DNA that was needed and diluted in distilled

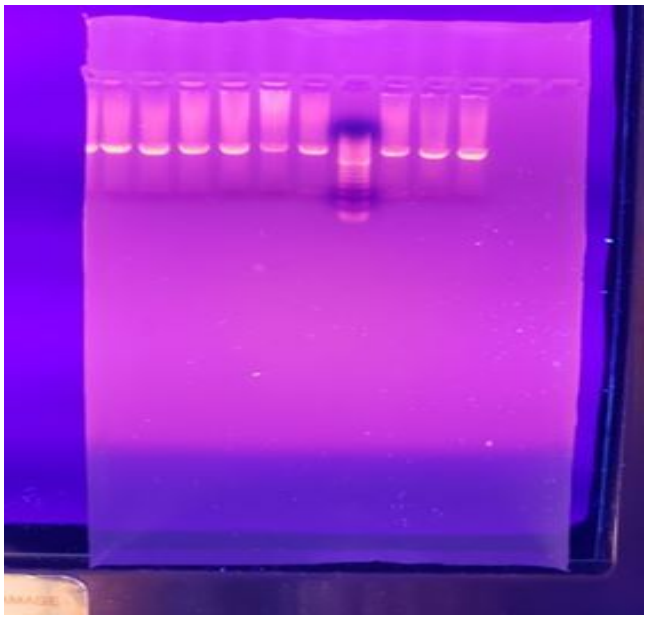
water, calculations were done with the concentration in order to send a 30µl sample for sequencing.

When the sequencing was completed and sent back the data received was checked and assessed through the BLAST database and compared to known bacteria to determine what the samples were.

Differences At Ramapo:

For timing purposes and in order to make sure that the sample were able to be identified, the samples were prepared for DNA sequencing. In order to prepare the samples for sequencing, an individual colony was taken from each of the Second Isolation plates. Each colony was inoculated in its own microfuge to with distilled water. After the sample was inoculated, the sample was then spun down multiple times so that the sample was properly mixed in with the distilled water. The Master Mix used for PCR followed the same recipe as that of Glasgow.

The sample underwent PCR, and then this sample was tested using DNA Gel Agarose and the results were the following:



As can be seen by the figure above, the results of all of the samples that underwent PCR were clear and visible. The lane that is uneven with the others, Lane 7 is the ladder.

Tests were done afterwards in order to further identify the samples and compare the results to the DNA sequenced results. One method of testing that was performed was for the purpose of seeing the rate at which bacteria growth occurred for select bacteria. The select bacteria were chosen out of the ones identified. Dilution plates were made using TSB liquid media, then the samples were left in an incubator at 37°C for growth. The chosen samples that were looked at for growth were looked at at temperatures of 4°C, 25°C, 37°C, 45°C, and 55°C, each with two plates per sample for each temperature. Two plates per sample per temperature were used in order to make sure the results were accurate and it is known that the test works similarly twice.

Results:

55°C				
Samples:	Endospore Stain	MacConkey	EMB	Mannitol
UVhd2 yellow	+	-	/	-
Kettle Red	+	-	/	-
Micro 1-1 yellow	+	-	-	-
Steamer (outer) yellow	+	-	+	-
Bottom left burner	+	-	-	+
VUVhd1	+	-	+	-
UVhd2 large	+	-	+	-
Vent hd2	-	+	+	-
Bathroom faucet white	+	-	+	-

Bottom of a pot	+	+	-	-
Micro white	+	-	-	+
Steph micro	+	-	-	-
Davidson orange	+	-	-	-
Davidson red	+	-	-	-
Davidson yellow	+	-	-	-
Davidson white	+	+	-	-

Table 1: Endospore Staining and differential Media testing for bacterial samples at 55°C

45°C				
Sample:	Endospore Stain	MacConkey	EMB	Mannitol
UVhd2 yellow	+	-	/	-
Kettle red	+	+	/	-
Micro 1-1 yellow	+	-	+	-
Steamer (outer) yellow	+	+	/	-
Kettle	-	+	/	-
VUVhd1	-	-	-	+
UVhd2 large	-	-	-	+
UVhd2 small	-	-	/	+
Bathroom faucet yellow	-	-	/	+
BRB1	-	-	-	/
BRB4	-	+	+	/
BRB6	-	+	+	/
Davidson red	-	+	+	-

Davidson orange	-	+	+	-
Davidson yellow	-	+	+	-
Davidson white	-	+	+	-

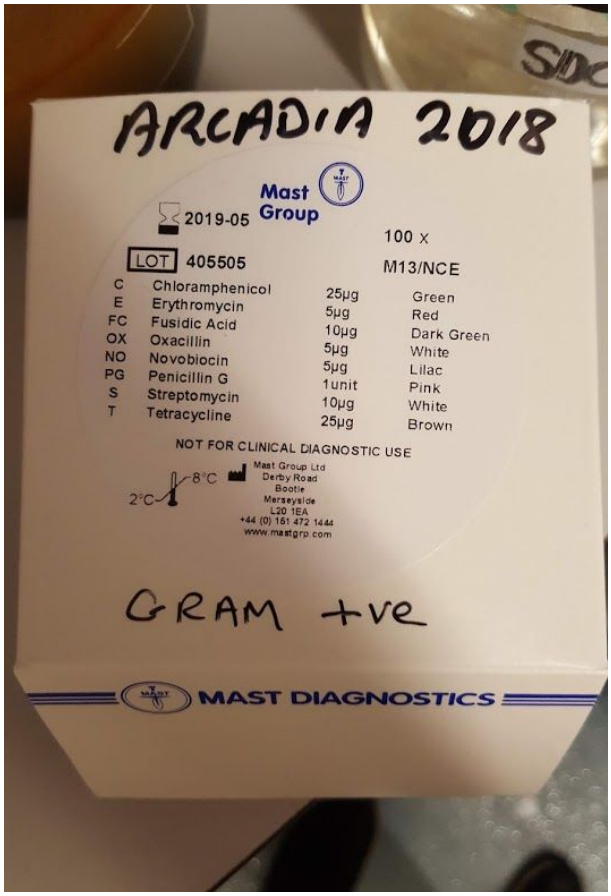
Table 2: Endospore Staining and Differential Media tests done at 45°C

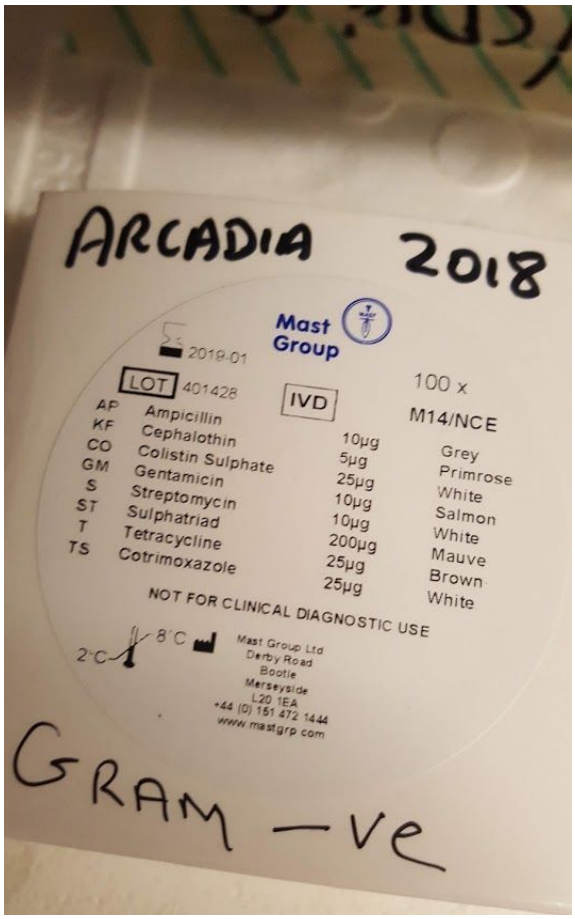
Endospore Staining at Higher Temperatures		
Sample:	60°C	65°C
Bottom left burner	-	-
UVhd2 large	+	+
BRB4	+	-
Bathroom Faucet White	+	+
VUVhd1	-	-
Microwave 1-1 yellow	-	-
Vent hd2	-	-
Kettle	-	-
UVhd2 yellow	-	-
Davidson yellow	-	+
Microwave white	+	/
Bottom of a pot	+	/
UVhd2 small	+	/
Steamer (outer)	/	+
Steph micro	-	+

Table 3: Endospore Staining done at 60°C and 65°C for samples that grew at those temperatures

In the table above, "-" means that the sample was endospore negative, "+" means that the sample was endospore positive, and "/" means that the sample showed no growth at one of

the two temperatures. If the sample did not grow at either temperature, the sample was not listed.





Figures 17 and 18: Lists of what each antibiotic is for Gram positive and Gram negative bacteria

M13 (gram positive) Antibiotic Assay								
Samples:	**C**	**E**	**FC**	**OX**	**NO**	**PG**	**S**	**T**
DMW	S	S	S	S	S	R	S	S
DMO	R	R	S	S	S	R	S	S
DMR	S	S	S	S	S	S	S	S
BFW	S	R	R	R	S	R	S	S
BFY	R	S	R	R	S	R	S	S

K	R	S	S	S	R	S	S	S
KR	R	S	S	S	S	R	S	S
Scoop	R	R	S	R	S	R	S	S
BLB	R	R	S	S	S	R	S	S
Steamer	R	S	S	S	S	R	S	S
M1W	S	R	S	S	S	S	S	S
M1Y	S	S	S	S	S	R	S	S
UVhd2s	R	R	S	S	S	R	S	S
VUVhd1	R	R	R	R	S	R	S	S
UVhd2L	S	S	S	R	S	R	S	S
Total S	6	8	12	10	14	3	15	15
Total R	9	7	3	5	1	12	0	0

Table 4: M13 Antibiotic Assay for Gram Positive Bacterial Samples

For both Tables 4 and 5, the “R’ signifies that the bacteria was resistant to the antibiotic, and the “S” signifies that the bacteria was susceptible to the antibiotic.

M14 (gram negative) Antibiotic Assay								
Sample:	**AP**	**KF**	**CO**	**GM**	**S**	**ST**	**T**	**TS**
BRB1	S	S	S	R	R	R	S	S
Bottom of Pot	R	S	S	S	S	S	S	S
UVhd2y	R	R	R	S	S	R	S	S
DMY	R	R	R	S	S	S	S	S
SM	R	R	R	S	S	R	S	R
BRB4	R	R	S	R	R	R	R	R
Vhd2	R	S	R	S	S	R	S	S
BRB6	R	R	R	S	S	R	S	S

Total R	7	5	5	2	2	6	1	2
Total S	1	3	3	6	6	2	7	6

Table 5: M14 Antibiotic Assay for Gram Negative Bacterial Samples

Gram and Endospore Stains at 37°C		
Sample:	Gram Stain	Endospore Stain
BRB1	-	-
BRB4	-	-
BRB6	-	-
VUVhd1	+	-
Vent hd2 large	-	-
Gross electric kettle	-	-
Bathroom faucet white	+	-
Bathroom faucet yellow	+	-
UVhd2 small	+	-
UVhd2 large	+	+
Davidson red	+	-
Davidson orange	+	-
Davidson yellow	-	-
Davidson white	+	-
Steph microwave	-	-
UVhd2 yellow	-	-
Kettle red	+	-
Steamer (outer) yellow	+	-
Bottom left burner	+	-
Microwave 1-1 white	+	+

Microwave 1-1 yellow	+	-

Table 6: Gram Stain and Endospore Stains for Bacterial Samples at 37°C

37°C				
Sample:	Mannitol	EMB	MacConkey	SAB
BRB1	/	-	-	**A**
BRB4	/	-	-	**G**
BRB6	/	-	-	**B**
Vhd2	-	-	-	**B**
VUVhd1	+	-	-	**A**
Bathroom Faucet yellow	/	/	/	**B**
Bottom of a pot	+	/	+	**B**
UVhd2 small	-	/	-	**B**
kettle	/	-	/	**A**
Bathroom faucet white	/	-	-	**A**
Uvhd2 large	-	/	-	**A**
Microwave 1-1 yellow	/	/	-	**B**
Microwave 1-1 white	-	/	-	**G**
Steamer (outer) yellow	/	/	-	**A**
Bottom left burner	/	/	+	**A**
UVhd2 yellow	-	/	-	**B**
Kettle red	/	+	+	/

Davidson white	**-**	**/**	**+**	**A**
Davidson yellow	**-**	**/**	**+**	**G**
Davidson orange	**/**	**/**	**-**	**B**
Davidson red	**/**	**+**	**+**	**/**
Steph microwave	**/**	**/**	**+**	**B**
Scoop	**+**	**/**	**+**	**/**

Table 7: Differential Media for Bacterial Samples at 37°C

For Table 7, "/" signifies that there was no growth on that plate. The "+" signifies that there was positive fermentation, and the "-" signifies the negative fermentation. "A" signifies that the bacteria partially attacked the blood cells in the agar plate, "B" completely attacked blood cells in the agar. "G" shows that the bacteria grew over the sample but did not attack a blood samples.

Sample Name:	Bacteria Identified:
BRB1	*Leptothrix*
BRB4	*Stenotrophomonas maltophilia*
BRB6	*Cupriavidus*
BP	*Staphylococcus haemolyticus*
BLB	*Dermacoccus nishinomiyaensis*
BFW	*Burkholderia mallei*
BFY	*Sphingomonas paucimobilis*
DMW	*Micrococcus luteus*
DMY	*Kocuria*
DMO	*Dermacoccus nishinomiyaensis*
UVL	*Brevibacillus brevis*
UVY	*Kocuria*

VUV	*Bacillus licheniformis*
M1W	*Staphylococcus haemolyticus*
M1Y	*Dermacoccus nishinomiyaensis*
K	/
SM	*Dermacoccus nishinomiyaensis*
SY	/
Vhd2	/
UVS	*Staphylococcus haemolyticus*

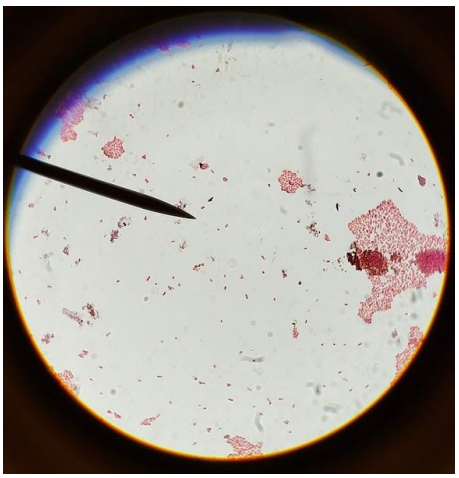
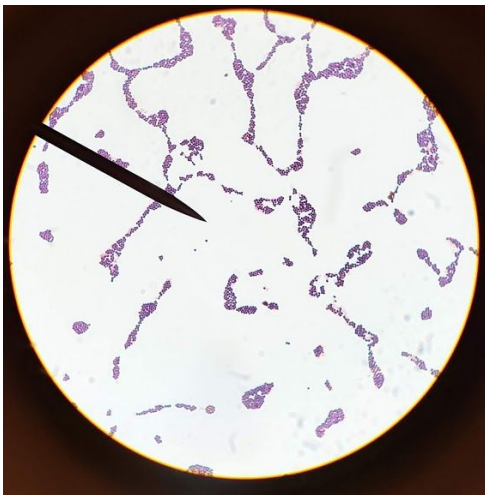
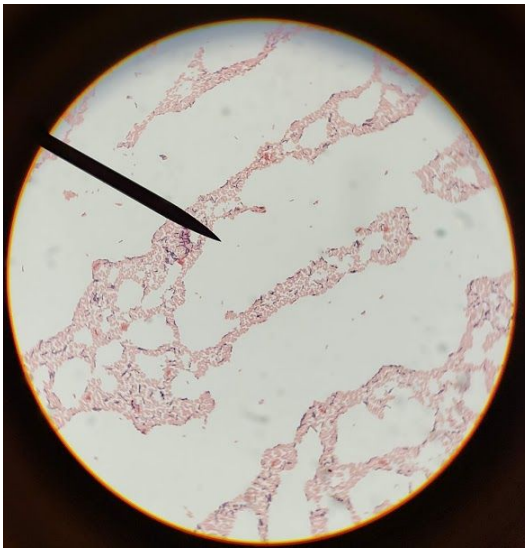
Table 8: List of names of the bacterial samples sequenced in UK

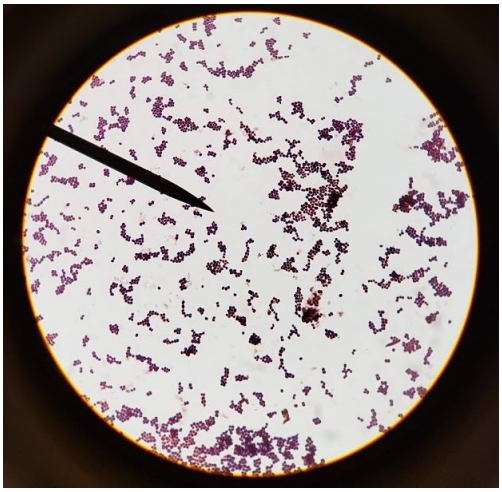
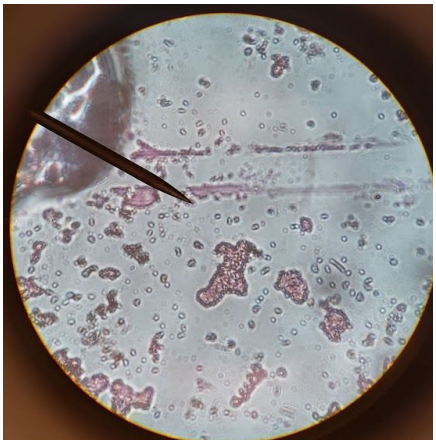
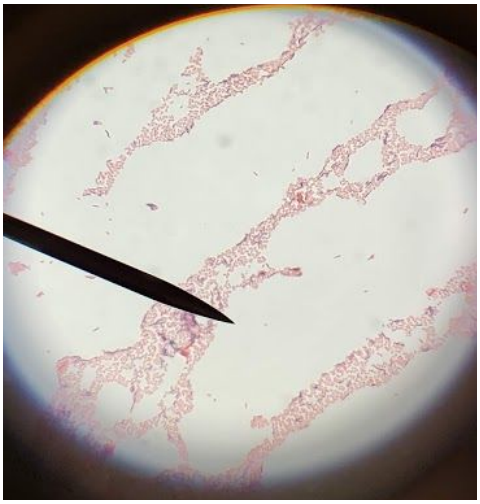
Sample:	Bacteria Identified:
F2SY	*Acinetobacter radioresistans strain K2NRBAO003*
F1	*Chryseobacterium taihuense strain AMY_6.1.1*
F2W	*Klebsiella pneumoniae strain J41*
HDRG	*Staphylococcus epidermidis strain JST6*
LV1	*Chryseobacterium takakiae strain ab5*
LV2	*Staphylococcus hominis strain PRF50*
M410	*Bacillus niabensis strain F5.9*
PHC	*Staphylococcus haemolyticus*
NYPF	*Pantoea agglomerans strain Ns13*

Table 9: List of names of the bacteria samples sequenced in U.S.

Samples were sequenced and identified prior to any testing due to reaching a deadline for DNA samples to be sent out to be sequenced.

Sample name:	Gram Result:
F2W	Gram negative (-)

	
PHC	Gram positive (+) 
NYPF	Mixed culture of both gram positive and negative* (+/-) 

LV2	Gram positive (+) 
F2SY	Gram negative (-) 
M410	Gram negative(-) 
F1	Gram positive cocci(+)

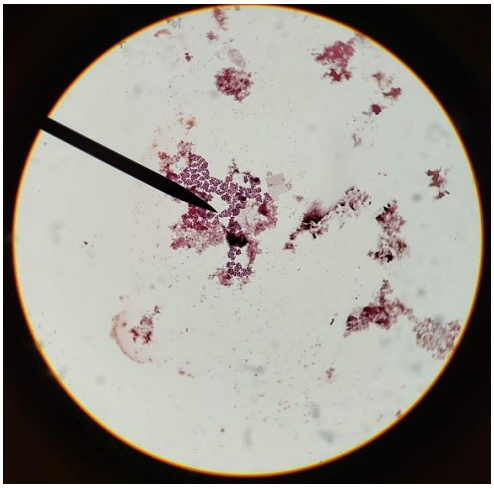
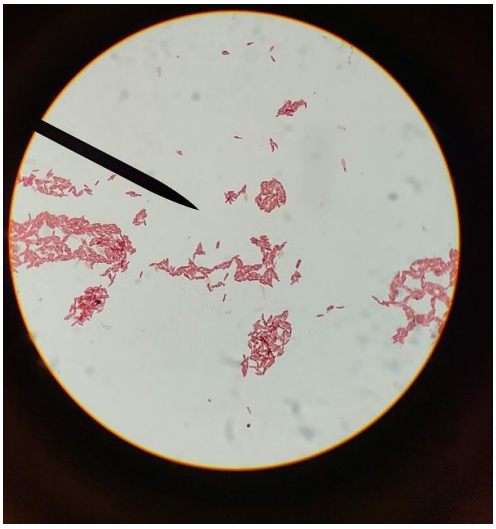
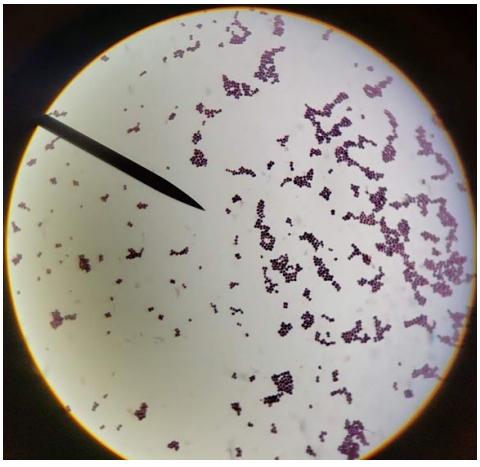
	
HDRG1	Gram negative(-) 
LV1	Gram positive cocci (+) 

Table 10: Tests on Gram Staining for all samples found in the US

Dilution plates for M410 over the course of two days					
Day 1			Day 2		
Temperature:	Colonies (plate #1)	Colonies (plate #2)	Temperature:	Colonies (plate #1)	Colonies (plate #2)
4°C	0	0	4°C	0	0
25°C	43	32	25°C	200	300
37°C	720	730	37°C	800	900
45°C	750	900	45°C	700	1000
55°C	1	220	55°C	10	280

Table 11: Growth using Dilution plates on sample M410

Discussion:

When looking at the results of each trial and test, many things can be understood. Table 8 shows the list of all of the different bacterial species that were collected and able to be sequenced. To get a better understanding of all of the bacteria, the testings were done prior to the sequencing so that there would be records of how each of the bacteria functions. The main hypothesis for this experiment was to see if endospores form at higher or lower temperatures in order to determine if the bacteria found are thermophiles or mesophiles in the urban environment they were found in.

For the bacteria found on the Boiler Rack of Bench 1 (BRB1), the bacteria found is known as *Leptothrix*. Literature states that it is gram negative, which goes along with the information in Table 6. This bacterium never grew to show spores at any temperature, out of the ones tested (37°C - 65°C), so it can be assumed that this is a non-spore forming type of bacteria. The bacteria also was gram negative, and for the MacConkey and EMB agar plates

the bacteria was not lactose fermenting. Looking further in, Leptothrix is an oxidizing bacterium, especially for Fe and Mn, and is typically found in an aquatic environment (Kunoh et al, 2015). Since this was a sample taken from the side of the Boiler Rack on Bench in a lab setting, this makes perfect sense. The inside of the Boiler Rack is metal, and water is always left in the Boiler Rack. Overall this bacterium can be determined that it is not a thermophile or mesophile, and most likely non-endospore forming, because the sample only grew at 37°C and 45°C, and did not grow any endospores, and did not grow at any higher temperatures.

Stenotrophomonas maltophilia was found to be the bacteria isolated from Boiler Rack 4 in the laboratory in which the study took place. It is gram negative, and highly resistant to antibiotics, only being susceptible to Colisten Sulphate (CO) out of all of the antibiotics tested. This bacteria grew between 37°C and 60°C, with endospores forming at 60°C, and the bacteria did not grow at 65°C, so the optimal temperatures of growth are between 37°C and 55°C, indicating that this is a mesophilic species. This bacterium is uncommon and usually found in humid environments, typically catheters, and once it enters the body it can cause Urinary Tract Infection, Respiratory Tract Infection, Bacteremia, and Endocarditis (Brooke et al, 2012).

The bacteria found in Boiler Rack 6 was determined to be *Cupriavidus*. It is gram negative and relatively harmless, in that it is susceptible to all but one of the antibiotics tested for, which is Ampicillin (AP). The bacteria grew at all of the temperatures tested up until 60°C, and at 65°C stopped growing. Endospores formed at 55°C and 60°C, signifying this bacteria is more comfortable in an environment at least at 37°C. This bacteria is only growing at 37°C and 45°C, with no endospores forming, and showed no lactose or mannitol fermentation. With a lot of attacking of the blood cells in the Sodium Azide blood (beta) agar as well, this bacteria can be determined to be only somewhat harmless, only attacking those who are already immunodeficient in most cases (Kobayashi et al, 2016). So this is not a thermophile or

mesophile, and is generally found to be harmless in most cases.

The bacteria found on the outside of a saucepan (BP) , a UV light on a UV hand dryer in a restaurant (UVS), as well as on a phone battery pack (PHC) is known as *Staphylococcus haemolyticus.* It is gram negative and multidrug resistant, resistant to Ampicillin, Cephalothin, Colisten Sulphate, and Cotrimoxazole. It is an invasive and opportunistic pathogen, often found on skin and medical devices in a clinical setting, and once a wound opens, the bacteria will attack the blood. It grows from 37°C to 60°C, and both samples showed this bacteria to be Beta for sodium blood agar, showing there was a lot of attacking of the blood (Czekaj et al, 2015). This bacteria when infecting humans causes sepsis, staph infection, bacteraemia, and peritonitis (Neu, 1994). Since it grows well at 37°C to 55°C, and then gets endospores at 60°C, and the fact that this bacteria grows well at the human body temperature shows that is is likely a mesophile.

An interesting thing to note from this is that the same bacteria (*Staphylococcus haemolyticus)* was found in areas of Glasgow as well as the United States. Though it could become a dangerously serious threat in the event that humans are infected with it, it can be inferred that this bacteria is a widespread and common species, and can survive in a decently wide range of temperatures and environments.

Dermacoccus nishinomiyaensis is found in the microwave of one of the research professors at their home (SM), the bottom left burner of a stove in a student flat (BLB), as well as another microwave in a student flat (M1Y). It is shown to have both gram negative and gram positive results, literature says it is positive, showing resistance to either one of some of the antibiotics, but not many. They all grew at all the temperatures tested, and endospores formed for two of the samples out of three at 65°C, supporting this is a mesophile and maybe even a thermophile.

In the bathroom faucet of a student flat in Glasgow (BFW), *Burkholderia mallei* was found. It is gram-positive, multidrug resistant, resisting 4 of the 8 drugs tested for, and grows at all temperatures, with endospores forming at 60°C and 65°C, so this is at least a mesophile and possibly a thermophile. It attacks blood, as seen on the sodium azide agar plate (alpha), and is known for causing two main diseases: glanders and melioidosis. It more usually effects horses and animals, but humans can be infected through contact with an infected animal (Sarkar-Tyson et al, 2009).

Sphingomonas paucimobilis is a gram-negative bacteria found in the bathroom faucet of a student flat that does not ferment any differential media. It can cause huge problems in the clinical settings, contaminating many things and entering the blood of humans, causing infection and bacteraemia outbreaks (Maragakis et al, 2009). It is antibiotic resistant to four of the eight bacteria tested. It is not a thermophile since it does not grow past 45°C on the agar plates that were incubated.

Micrococcus luteus was found in a communal microwave in an academic building at a university. It is gram positive and also susceptible to all but Penicillin G (PG). The sample grew up until 55°C, forming endospores only at 55°C then not growing in any temperatures past that, so this is not a thermophile, but is a spore forming bacteria when necessary. They have a stricter ecological niche in that they are only found on mammal skin (Young et al, 2010).

Kocuria was found in both a communal microwave in an academic building and a UV hand dryer at a restaurant. It is a gram-positive bacteria that is able to grow at every temperature tested. There were no endospores at any temperature, so it could be that the bacteria is non-spore forming, or it is able to grow even higher in temperature with a huge range in temperature it is able to work well in, so it is most likely a mesophile because it was able to grow up to 65°C. This is a non-pathogenic bacteria rarely associated with human infection, but

this bacteria is also multidrug resistant if one were to be infected, so it would become an issue then (Kandi et al, 2016).

Brevibacillus brevis was determined to be the bacteria that was found in the UV light hand dryer at a local restaurant. It is gram positive and resistant to oxacillin and penicillin G. The bacteria grows at all of the temperatures, but endospores form at 55°C, 60°C, and 65°C, signifying those temperatures are not optimal for growth. The antibiotic assay showed results of only resisting two antibiotics. This bacteria is known for its high heat tolerance, as well as assisting in plant growth (Nehra et al, 2016), but not as much for infection or harm to humans, so this bacteria is not one of concern.

Bacillus licheniformis is a bacteria that was also found in a local UV hand dryer in a restaurant. This bacteria is gram positive, and grew at all temperatures. The endospores though, actually were positive at lower heat levels. At 37°C and 45°C, there were endospores formed, but as the incubator rose to hold them in at higher temperatures, the endospores were no longer there for 55°C, 60°C, or 65°C. This indicates that this sample maybe have been thermophilic, in that it is a lot more comfortable in hotter environments than even human body temperature. There are other great feats in research being done in that this bacteria could be the key to making the feathers edible and make food supply cheaper.

Bacillus niabensis was the sample found in the inside of a microwave in a dormitory at a Ramapo College. They are rod shaped, gram positive bacteria. This bacteria grew at 25°C, 37°C, 45°C and at 55°C, which is every temperature that was tested. Since this bacteria grew at all temperatures there is indication that it is heat resistant. Through online research for more details on this species, this type of bacteria is typically a spore forming type when it gets to high temperatures. Looking at previous research, *Bacillus niabensis* was determined to be mesophilic (Kwon *et al*, 2007). This species also forms in chains. In the dilution series

experiment, out of the three samples tested using this method, this was the only sample to grow, and it grew at every temperature aside from 4°C. This sample grew the most colonies on the plates that were incubated at 45°C. It was also worth noting that the sizes of the colonies were larger at 45°C in addition to there being more colonies. From these results, this implies that the optimal temperature is close to 45°C.

Normally, *Pantoea agglomerans* is a gram negative bacteria, but sample resulted in a mixed culture. This is due to using the colonies from the first isolation rather than the second isolation plate for the sample. *Pantoea agglomerans* was able to grow successfully at 25°C, 37°C, and little growth at 45°C. This indicates that the optimal temperature is somewhere between common room temperature and 37°C, which is the common human body temperature. Since it grew at temperatures between 25°C and 45°C this is the most common range of temperatures for mesophiles, so it can be determined to be mesophilic as well. This bacteria is said to be commonly found inside the digestive tracts of various insect species, such as mosquitos. In mosquitos particularly, this is a symbiotic bacteria that assists in fighting off the disease Malaria (Wang *et al,* 2012), as well as in human fecal matter. This sample was found on the floor of New York Penn Station, near a heater in the winter.

Staphylococcus hominis was found at the bottom of a laptop, near the vent area that is used for cooling the laptop when it overheats. This sample presented itself as gram positive in cocci shape bacteria. The temperature at which there was the most growth was at 37°C, with growth at 25°C and 45°C as well. This makes sense because the most common place for this bacteria to be is on the skin, nail, and hair of humans. This bacteria is typically harmless to those with normal immune systems. This was tested on Mannitol Salt agar, and had a negative result, which shows that it is not pathogenic.

Chryseobacterium takakiae was found near the vent of an overheating laptop. *Chryseobacterium taihuense* was found on the spout of a faucet in a dorm at Ramapo College. Both of these bacteria have similar traits and found the same data from the two of them. The two were both found to be Gram positive cocci shaped bacteria. They did not grow past 37°C, and grew better at 25°C. Looking into these bacteria through use of search engines, *Chryseobacterium takakiae* is a very specific bacteria that is found in the plant Takakia lepidozioides, a small plant that grows in colder climates (Zhao *et al*, 2015). When tested on a Mannitol Salt Agar, the specimen F1 was determined to be non-pathogenic. These particular samples found were actually classified as cold resistant bacteria rather than heat resistant. This makes sense since it is found in colder climates and areas of higher altitudes (Zhao *et al*, 2015). These samples specifically were tested at 4°C because of this, but they showed no growth over 1 week at 4°C. Further testing can be done in a study of cold resistant bacteria.

Staphylococcus epidermidis is commonly found on the hair, skin and nails, similar to *Staphylococcus hominis.* It is one of the most common non-pathogenic forms of *Staphylococcus,* and is gram positive cocci shaped, similar to the other types of *Staphylococcus* found. This bacteria was found in the hand dryer at Ramapo College. It grew at 25°C, 37°C, and 45°C, with the most growth at 37°C. Having optimal growth at the same temperature as the human body temperature makes sense considering that is where this bacteria is most commonly found. It makes sense as well that this was found in a hand dryer, something that blows air onto the human skin and nails.

Klebsiella pneumoniae is listed as one of the more pathogenic types of bacteria. According to the Center for Disease Control, the bacteria can cause bloodstream infection if it enters the blood, or pneumonia if the bacteria enters the lungs. This bacteria easily spreads through the air and from person to person contact, especially in hospital settings. This bacteria

was found on the spout of one of the faucets in the dorms at Ramapo College as well. This bacteria was found to be gram negative. This bacteria grew at 25°C, 37°C, 45°C, and 55°C, which is a wide range of temperatures.

Acinetobacter radioresistens is a particular bacteria that piqued my interest. The bacteria is radiation resistant, and shows resistance to ionization radiation. Ionizing radiation is the forms of radiation that have a higher frequency, such as Gamma rays, X-rays, or microwaves. This sample was found in a 1100W microwave at Ramapo College. Since this radiation resistant bacteria is found in a microwave that uses ionization radiation, this further proves the idea that bacteria are evolving along with urban advancing technology. Not only are the heat resistant bacteria becoming more suitable for high temperature environments, or the cold resistant bacteria adapting to stay in cold areas such as water spouts, but bacteria are even able to survive in something with as much high frequency radiation as a microwave, to the point where it can live comfortably. This bacteria was found to have gram negative bacteria, and was able to grow on plates incubated at 25°C, 37°C, 45°C, and 55°C, showing a wide range of temperatures at which it can do well in. This bacteria was attempted at in trying dilution plates, but the results were showing no growth.

Overall, the bacteria can be understood better and feel like less of a threat when knowing there is always at least one thing that the bacteria is resistant to (out of the ones tested). Since sterilization methods as of nowadays are not the greatest, with all the heat tolerant bacteria, new methods of sterilization must be put in place. In places such as Scotland, instillation of radiation with UV lights in hand dryers are put in place as a newer means of sterilization, but with the amount of various types of bacteria found on the lights themselves it does not prove to be a satisfactory means of improving sanitation. The lights were instilled in order to have radiation and heat treatment to kill bacteria that develop in hand dryers, but if

bacteria are growing on the lights themselves, there are obvious problems. Radiation resistance in certain forms of bacteria is also necessary to take into account when viewing means of new ideas on how to sterilize objects and keep safe from certain types of harmful bacteria. Kettles and boiling racks were also tested and found to have bacteria as well, so there are bacteria that are able to survive even boiling temperatures. Future studies should go into theorizing what will kill each type of bacteria, and further develop sterilization methods that would be suitable for the ever-evolving bacteria that could harm humans. In future studies, testing areas of warmer temperatures where the bacterial samples would be very beneficial to the study, or collecting samples in the summertime.

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