5-16-2020

Phenotypic Analysis of S. aureus strains from University Student Health Centers and Environmental Controls
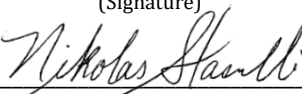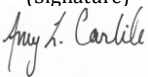
Antonio Teixeira

UNIVERSITY OF NEW HAVEN
HONORS PROGRAM

2019-2020 Honors Thesis

Phenotypic Analysis of *S. aureus* strains from University Student Health Centers and Environmental Controls

Antonio Teixeira

A thesis presented in partial fulfillment of the requirements of the Undergraduate Honors Program at the University of New Haven.

Student: _____
(Signature)

Thesis Advisor: _____Nikolas Stasulli_____
(Signature)

Department Chair: _____Amy L. Carlile_____
(Signature)

Honors Program Director: _____
(Signature)

05.16.2020
_____
Date

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University Student Health Centers and Environmental Controls

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Honors Thesis - Biology

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Faculty Advisor: Nikolas Stasulli

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a group of pathogenic bacterial strains resistant to a class of antibiotics that is a major cause for concern in health care systems. There is a lack of study in whether health care centers are reservoirs for these bacteria, especially within developed countries. Five environmental swab samples were collected from five different university health care centers across the region, and 16 swab samples from a general university environment. Any isolated bacterial strains collected underwent five biochemical tests (mannitol fermentation, DNase activity, oxidase activity, catalase activity, and coagulase activity) to preliminarily identify *S. aureus* bacteria. 24% of the clinical samples and 25% of environmental samples contained *S. aureus*, signifying an equal distribution of the species among the two location groups. In addition, extensive literature review showed how isolated *S. aureus* strains can easily be characterized as MRSA, through antibiotic disc-diffusion testing and genetic sequencing of the potential *SCCmec* region. This genetic sequencing can also identify *SCCmec* type and class, identifiers useful in comparison to current MRSA studies.

Introduction

Staphylococcus aureus is a species of bacteria known to cause infection, primarily through its introduction into, and incubation within, an open wound. This bacterial species was one of the earliest to be heavily studied in the medical community, largely due in part to its susceptibility to penicillin. However, greater exposure and treatment with these newly discovered antibiotics allowed *S. aureus* to quickly acquire antibiotic resistance (Centers for Disease Control and Prevention, 2020). Antibiotic resistance is a phenomenon in which the few bacteria resistant to the specific therapy can survive and multiply after treatment. The resistant bacteria can then cause disease in a new patient, who cannot be treated with the same antibiotic or therapy as the first patient. Over time, all new infections from that bacterial species will be immune to a specific therapy.

As a result of increasing antibiotic resistance, *S. aureus* quickly became resistant to the first commercially viable antibiotic, penicillin, by the 1940s, and to the antibiotic's subsequent synthetic alternative, methicillin, by the 1970s (National Institute of Allergy and Infectious Disease, 2016). These strains which are resistant to β-lactams, the antibiotic family of methicillin and penicillin, are known as methicillin-resistant *Staphylococcus aureus* (MRSA). Recently, extensive studies have shown that an increasing number of MRSA strains are showing resistance to a more aggressive antibiotic, vancomycin, highlighting the increasing resistance of these pathogens (Hasan, Acharjee, & Noor, 2016). Understanding whether or not these bacteria are present in health care treatment centers, along with their characteristics, will allow medical staff to prepare for, and combat, the growing wave of antibiotic resistance.

In traditional healthcare settings, bacterial infections are primarily transmitted through direct contact, such as touching a contaminated surface (fomite) and then immediately touching an open wound. Other routes of infection include close contact or quarters with areas soiled by human waste (Center for Health Protection, 2017). MRSA can cause severe infections, such as bacteremia, sepsis, pneumonia, and even death. Even with the initiatives to prevent transmission, MRSA infections are persistent in healthcare settings.

Current MRSA Studies

One large area in which MRSA samples are analyzed from environmental sources are livestock animal populations. *S. aureus* has been shown to infect humans through ingestion of animal products (meat, poultry, dairy, etc.), but only in rare cases. Animals are mainly used as a sample population to investigate the effects of antibiotic therapies on *S. aureus*. Giacinti et al. (2017) isolated MRSA in dairy sheep from farms in central Italy. The researchers found that while the *S. aureus* presence in the samples collected was high (53.3%), only 0.7% of samples had MRSA. While the bacteria have been shown to transmit illness through livestock and their subsequent dairy, meat, or poultry products, the most common and lethal method of transmission is through contamination in healthcare centers. (Centers for Disease Control and Prevention, 2017)

Another large area of study with the MRSA bacteria is in antibiotic resistance testing using samples from patients, often directly collected from the wounds of patients. These wound isolation studies are primarily done to test the effectiveness of new antibiotic therapies. One recent example of this type of study, performed in Turkey, used samples from patients diagnosed with and treated for MRSA. The researchers were using the patient isolates to test the efficacy

of a new antibiotic, clindamycin (Gul et al., 2008). The researchers found that 64.1% of the isolates were resistant to clindamycin, and that 84.9% were resistant to erythromycin, an antibiotic of the same class.

A third, and least common, type of study involving MRSA utilizes environmental isolates collected from hospitals. Abdolmaleki, Mashak, and Dehkordi (2019) isolated MRSA from cockroaches captured in hospitals, both from skin scrapings and from gut dissections. After isolation for MRSA, the bacteria were characterized phenotypically and genotypically to observe the defining characteristics within the strains analyzed. They concluded that a high prevalence of MRSA was found, which were largely resistant to a multitude of antibiotics (penicillin, ceftaroline tetracycline, gentamicin and trimethoprim-sulfamethoxazole) and had a large number of genes characteristic for antibiotic resistance.

Considering that little research has been published on MRSA within health care facilities or on analysis of these bacteria from easily accessible environmental surfaces, the current study seeks to analyze *S. aureus* strains from accessible health care centers in the greater New Haven region.

Phenotypic Characteristics of MRSA

As touched on above, the testing of antibiotic resistant MRSA strains is typically performed in a research setting. This is done for multiple reasons. Firstly, it is to confirm their resistance against methicillin. However, laboratories typically use a substitute β-lactam antibiotic (such as oxacillin or cefoxitin) instead of methicillin due to their longer shelf-life and lack of commercially available methicillin. Secondly, testing of antibiotics outside of the β-lactams allows for researchers to determine if MRSA strains are acquiring additional resistivity. Of specific

interest is novel resistance to antibiotics with a different mechanism of action. Figure 1 in the Appendix shows characteristic antibiotics of each mechanism of action.

In a 2015 study, Chang, Dhaliwal, Raju, and Kowalski (2015) tested wound cultures from various hospital patients, isolated from January 1993 to November 2012. MRSA strains were shown to be the most susceptible to the following antibiotics at the following percentages: sulfamethoxazole (94.3%), bacitracin (89.3%), trimethoprim (88.5%), and gentamicin (86.1%).

In their study of clinically collected bacterial samples from patient wounds in Bangladesh, Parvez, Ferdous, Rahman, and Islam (2018) tested the *S. aureus* bacteria for antibiotic resistivity. Results are as follows: oxacillin (97%), gentamicin (85%), ceftazidime (76%), tetracyclin (68%), chloramphenicol (66%), cirproflxacin (53%), and clindamycin (34%).

Kateete, et al. (2019) tested *S. aureus* from healthy Ugandan children for antibiotic resistivity. All MRSA isolates were susceptible to vancomycin, linezolid, and clindamycin. Resistances are as follows: trimethoprim-sulfamethoxazole (73.3%), erythromycin (75.6%), chloramphenicol (60%), gentamicin (55.6%), and ciprofloxacin (35.6%).

Genotypic Characteristics of MRSA

In addition to phenotypic analysis, a bacteria's genome can be utilized to help determine its resistivity. The presence of a gene can be indicative of a bacterial strain's resistance to antibiotics, as the gene would encode for a protein which can combat the antibiotic's effects on the cell.

MRSA strains can produce antibiotic resistance against a certain family of antibiotics, known as β-lactams, through an altered penicillin-binding protein. This altered protein has a decreased affinity for most β-lactam antibiotics, as the active site conformation is not conducive

to the characteristic molecular structure of these chemicals. The penicillin-binding protein (PBP) is encoded by an acquired gene, *mec*. This gene is carried on the mobile staphylococcal cassette chromosome *mec*, or *SCCmec* (Katayama, Ito, & Hiramatsu, 2000).

SCCmec elements are classified into a system of types and subtypes, defined by the *mec* gene complex and *ccr* gene complex. A gene complex includes its defining gene and other genetic elements related to the function and transcription of the gene. While the *mec* gene complex is responsible for β-lactam resistance, the *ccr* gene complex is responsible for mobility of the *SCCmec* within and between genomes.

The *ccr* gene complex may contain three distinct genes: *ccrA, ccrB,* and *ccrC.* Each gene has its own allotypes, variants of the original with only small differences that do not have a large impact on its function. Figure 2 shows the relationship between the *ccr* gene variants.

The *ccr* complex is typed according to which genes are present. Types 1-4 carry one *ccrA* and one *ccrB* gene and are distinguished by which allotype is present, while type 5 carries only *ccrC* gene(s). (International Working Group on the Classification of Stphylococcal Cassette Chromosome Elements, 2009) The *ccr* genes can be detected by PCR analysis by utilizing primers specific to the gene/allotype in question.

The *mec* gene complex generally consists of the *mecA* gene and variants of other regulatory genes and insertion sequences. The variants of the gene complex are distinguished only by the surrounding genetic elements, and are distinguished into classes based on their presence, orientation, and length. The variants are identified as different classes: Class A, Class B, Class C, and Class D. (International Working Group on the Classification of Stphylococcal Cassette Chromosome Elements, 2009) There has also been a recently reported Class E, which

contains the *mecC* gene in place of the *mecA* gene. Figure 3 shows the classes of the *mec* gene complex, along with some sub-classes.

The *SCCmec* region is a transposable element, meaning it can be inserted within a genome and change its position. The vast majority of all known MRSA strains are inserted within the *orfX* gene, a gene conserved among all *Staphylococci* (Hiramatsu, et al., 2013). With MRSA strains, the *orfX* gene is essentially split, with the original genetic sequence conserved. From this, researchers can easily isolate, amplify, and analyze the entirety of the *SCCmec* region by knowing its preceding and succeeding genetic sequences (the split *orfX* genetic sequence).

Methodology

Clinical Sample Collection

Universities who were willing to participate in this study are the University of New Haven (West Haven, CT), Quinnipiac University (Hamden, CT), Sacred Heart University (Bridgeport, CT), Bridgeport University (Bridgeport, CT), and Trinity College (Hartford, CT). Universities are addressed by a randomly assigned number (Location 0, Location 1, etc.), so as to not link a specific school to isolated strains, and all other future results.

Five samples were gathered from a random patient exam room at each health center. Since room set up differed at each university, the samples were taken from and labeled as follows: two countertop or table samples, one sink sample, one floor or floor corner sample, and one exam bed sample.

All environmental swabs were performed over the course of two weeks, in mid to late October 2019. Samples were collected in the following manner. A sterile cotton swab was moistened in a 0.9% saline solution. Immediately after, it was swabbed on a sample collection area and placed into the micro centrifuge tube containing the saline. Due to cotton swab length, a portion of the swab stick was cut with a pair of sterile scissors, so that the cotton end could be secured within the tube. After all samples were collected from a location, the tubes were sealed in a plastic bag and stored in cool, refrigerated environments until transfer to the University of New Haven laboratory space.

All tubes were labeled after securing of the swab, in a way to ensure university anonymity from results. Two numbers were written on each tube. The first number corresponds to the location number assigned to the university. The second number corresponds to the sample

collection area from which it originated. Table 1 in the Appendix shows these number designations. For example, the floor sample from Location 3 was labeled with the two-digit number 34, and the sink sample from Location 0 was labeled with the number 03.

Environmental Control Sample Collection

In addition to the above clinical samples, swabs and samples were taken from various environmental locations around the University of New Haven's West Haven campus. Thirteen samples came from buildings and areas commonly found on a college campus that are regularly trafficked by student, faculty, and staff. Two additional soil samples were collected. All fifteen samples were collected within a two-day period in early February 2020.

The first thirteen samples from various buildings across campus were collected via the same procedure used for the clinical samples. After collection, the tubes were labeled with number 100-113, based on collection order. For the two soil samples, a 50mL Eppendorf tube was used to collect approximately 30mL of soil from each location. These were labeled as samples 114 and 115. Table 2 shows the number assignments for all environmental control samples.

The soil samples were prepared and diluted prior to initial mannitol salt agar (MSA) plating. First, 1g of soil was diluted in 10mL of 0.9% saline. This was deemed the original soil solution (hereby referred to as the undiluted solution). Aliquots from the undiluted solution were extracted to create five diluted solutions, 1:1, 1:2, 1:4, 1:6, and 1:10 dilutions by volume, respectively. Each solution was then transferred to a microcentrifuge tube labeled with the sample number and corresponding dilution concentration (0 for undiluted, 1:1, 1:2, etc.)

Identification of *S. aureus* strains

A loopful of inoculum from the saline solution of each micro centrifuge tube was streaked onto a portion of a Mannitol Salt Agar plate. After streaking of all samples, all MSA plates were incubated at 30°C for 30 hours. Those samples which produced no bacteria growth or little growth with no mannitol fermenting bacteria were excluded from all further testing.

One area of mannitol fermenting bacterial growth from each pertinent sample was streaked to single colony on individual MSA plates. For Samples 114 and 115 (the soil samples), one area of mannitol fermenting bacterial growth from only the 1:10 dilution was streaked to single colony. After 24 hours of incubation, a single mannitol fermenting colony was streaked onto another MSA plate. These final MSA plates were used to obtain bacteria for subsequent biochemical testing.

First, bacteria were streaked across DNase plates and incubated at 30°C for 20 hours. Plates were then flooded with a 0.1% Toluidine Blue solution and allowed to incubate again for 1.5 hours. Plates were examined for color change, indicating DNase activity. Next, bacteria from the final MSA plates were streaked across a microscope slide. A pure H_2O_2 solution was dropped on the slide, with any gas formation noted as catalase activity. Then, bacteria were streaked onto filter paper placed within a plate. The bacteria were then soaked with Remel's BactiDrop™ OXIDASE testing solution. Blue/purple color change was noted as positive oxidase activity, as per the manufacturer's instructions. Finally, tubes containing 0.3mL of 0.1% reconstituted rabbit serum were inoculated with sample bacteria and incubated at 30°C for 3 hours. Solidification/coagulation of solution within the tube was recorded as positive coagulase activity.

Results

Clinical Samples

Initial plating of the clinical samples revealed six mannitol positive bacteria from samples 04, 05, 11, 14, 23, and 45, all with moderate areas of growth. The rest of the nineteen samples in this group had no bacterial growth. Figures 4 – 10 show these initial MSA plates. Isolation to single colony for the six samples was successful, with clearly positive mannitol fermentation through each stage.

The first round of DNase testing for the six clinical samples yielded positive results. However, these results were not definitive due to poor execution of the plate flooding technique used to apply the pH indicator. After adjustment to the technique, the second round of DNase testing provided more definitive results. All six tested isolates showed clear pH indicator color change from blue to green-yellow, the sign of positive DNase activity. Figures 11 – 16 show these second round DNase plates.

Initial catalase testing of the six clinical samples were weakly positive, with only moderate to low bubble formation. Subsequent testing utilizing hydrogen peroxide stored in a dark environment provided the large bubble formation seen in Figures 17 – 19. All six samples were catalase positive.

Oxidase testing on the filter paper proved effective, with all six clinical isolates showing no color change to the reagent. Figures 20 – 22 show these results.

The two rounds of tube coagulase testing resulted in minimal clumping; the media did not fully solidify after incubation. Figures are not available due to presumed third round of testing (See "Shutdown" section of Discussion below).

Overall, six of the 25 samples from the clinical group were mannitol fermenting, and had subsequent biochemical testing fully consistent with *S. aureus* (DNase positive, oxidase negative, catalase positive, and coagulase positive).

Environmental Samples

Initial plating of these samples revealed three mannitol positive bacteria from samples 103, 106, and 112. There was only one large colony from samples 106 and 112, while sample 103 had many small colonies. In addition, all dilutions of samples 114 and 115 contained strong mannitol positive growth. These plates were colored yellow and fully scattered with bacterial colonies. Samples 100 – 102, 104, 105, 107 – 111, and 113 showed no growth on the MSA plates. Figures 23 – 34 in the Appendix show all initial environmental plates.

The first round of DNase testing again provided nondefinitive results due to the plate flooding technique. Figures 35 – 39 show the results from the second round of DNase plates after incubation with the pH indicator. The results from these were not ideal but show a clear color change with all six samples.

Again, catalase testing was initially weakly positive for all samples. Figures 40 – 44 in the Appendix shows these results. The second round of tests done with properly stored hydrogen peroxide resulted in abundant bubble formation for all five isolates.

Oxidase testing again proved effective with the environmental samples. Four strains were negative. Only the isolate from sample 114 was oxidase positive, with a clear purple change over the bacteria seconds after the addition of the reagent. Figures 45 – 49 show these results.

Tube coagulase testing for samples 103, 106, 112, and 115 were weakly positive with limited clumping after incubation. Sample 114 had no visible clumping. No figures were obtained due to another anticipated round of testing.

Overall

Six out of the 25 clinical samples contained mannitol-positive bacteria, all of which were likely *S. aureus* based on the available metabolic testing. Only samples from Location 3 yielded no bacteria growth on MSA plates. Five out of the 16 environmental samples contained mannitol-positive bacteria. Only one of these strains was not suggested to be *S. aureus*. The isolate taken from sample 114 was oxidase positive and coagulase negative. As it was catalase positive, it is most likely another *Staphylococcus* sp. but not of the species *S. aureus*.

24% of clinical samples contained *S. aureus* bacteria. 25% of environmental samples contained *S. aureus* bacteria.

Discussion

Obstacles

Initial timeline of the research was delayed, due to difficulties securing locations. Although university health care centers were not the primary target for sample collection, universities were the only institution willing to accept the proposal. The unanticipated additional contact hours resulted in an initial sample collection date of October 18th, 2019 instead of the originally planned early to mid-September window.

Results from initial sample streaking proved effective. Abundant mannitol positive bacterial growth was clear, even when limited to a fraction of the agar plate. This provided sufficient reasoning that lack of growth or mannitol positive growth in negative quadrants was not due to poor technique or potential bacterial competition.

The limited number of mannitol positive strains within the clinical isolates was unexpectedly low, as environmental studies within the field tend to yield high percentages of *S. aureus* isolates. This may be due to either good health center disinfection procedures or the disparity between first world health care procedures (the currently study isolates) and third-world health care procedures (where all current environmental isolate studies are done). Furthermore, the lower than anticipated *S. aureus* isolates from the environmental samples may provide an additional reason for the lower yield in the clinical samples. If there is less *S. aureus* in the environment than anticipated, then fewer of these bacteria can be transferred into the health centers. This is corroborated by the similar *S. aureus* percentage among both groups.

The DNase plate results were the most subjective results obtained. This was due to the inconsistencies that came with flooding the plates with pH indicator solution. Small pooling of

the solution on the plate resulted in areas ranging from blue to green on the plate. As such, an area of green color surrounding a section of bacterial growth was recorded as positive. Even after adjustments to combat this, including controlled pouring of smaller amounts of solution, level setting of plates, and use of a sturdy board within the grated incubation chamber, pooling can still be seen on some of the DNase plates photographed.

Oxidase tests could not be performed in duplicate due to a lack of time (see Shutdown section below). Once a procedure that produced clear results was found, the tests could not be duplicated before laboratory shutdown.

Coagulase test results were not photographed due to the shutdown, as well. After initial results for the environmental samples came back positive, preparations were underway to repeat the test for all mannitol positive samples (including those from the clinical group). It was this repeat test that would be photographed. Unfortunately, the laboratory shut down prior to the execution of the replicate coagulase test.

Shutdown

The University of New Haven decided on March 9th, 2020 to suspend all in-person learning activities and send all on-campus students home early, for a full 2 weeks, before the beginning of the scheduled Spring Break. On Monday March 16th, 2020, the University decided to move to remote learning for the remainder of the Spring 2020 semester. This included the closure of all on-campus laboratories.

For this reason, the original aims of this research could not be completed, which included further phenotypic testing of the isolated bacterial strains to determine antibiotic resistance and

preliminary genetic investigation of the bacteria. This was instead supplemented by additional literature review, especially in the genetics of MRSA bacteria (see Introduction above).

Planned Future Methodology

Firstly, completion of the replicate oxidase and coagulase testing on the 11 isolates would have taken place. Then, antibiotic resistance tests via disc-diffusion procedure would have been performed with six different antibiotics: Penicillin, Cefoxitin (a β-lactam antibiotic used in place of Methicillin for laboratory testing purposes), Vancomycin, Tetracycline, Erythromycin, and Sulfamethoxazole-Trimethoprim. Finally, DNA isolated from all *S. aureus* strains would have undergone PCR amplification with primers designed to target characteristic genes of the *SCCmec* region. Ideal primers would include those for the *orfX*, *mecA*, and *ccrC* genes. (Raji, et al., 2016) (Abdolmaleki, Mashak, & Dehkordi, 2019) (Hiramatsu, et al., 2013) Table 5 shows selected primers for this proposed PCR tetsing.

The sequencing of the entire *SCCmec* region would not be done, due to length of the region. Instead, a small region following the start of the *orfX* gene would be sequenced, to ensure that the transposable region is present in the bacterial genome. In addition, sequencing of any potential *mecA* and *ccrC* genes would be done, as these are the most common variants.

Overall, the planned testing would indicate whether the isolated *S. aureus* strains were methicillin resistant. The comparison of MRSA presence between the environmental control and clinical isolates would indicate whether the health centers were taking enough action in their disinfection and cleaning to prevent the transmission of the bacterial pathogen. In addition, MRSA findings from the environmental controls could provide further evidence of the prevalence of the bacteria within our society. The genetic test results would give some indication of the

pathogenicity of the strains isolated. In addition, they could provide some measure of relationship between the strains. A closer taxonomic relationship would be an indication of limited MRSA presence within the tested areas, as they are more likely to have come from the same initial bacterium.

Appendix

Explanatory Tables and Figures

Table 1: Labeling number assignments for each collection area

Sample Collection Area	Assigned Labeling Number
Countertop or Table - 1	1
Countertop or Table - 2	2
Sink	3
Floor / Floor Corner	4
Exam Bed	5

Table 2: Labeling number assignments for all environmental control samples

Sample Collection Area	Assigned Labeling Number
Dining Hall Plate	100
Dining Hall Tables	101
Dining Hall Silverware	102
Dining Hall Floor	103
Dormitory Door Handles	104
Dormitory Floor	105
Classroom Floor	106
Classroom Desk	107
Classroom Computer	108
Library Desk	109
Library Computer	110
Library Floor	111
Outdoor Cement Walkway	112
Student Lounge Furniture	113
Soil Sample 1 – Heavily Trafficked Mulch-Based Soil	114
Soil Sample 2 – Low Trafficked Vegetated Land	115

Table 3: Results of Biochemical Tests for all clinical samples, with *N/A* indicating that the test was not performed with the corresponding sample and multiple symbols indicating the results for all replicate tests done

Sample Number	Mannitol Fermentation (+/-)	DNase Activity (+/-)	Oxidase Activity (+/-)	Catalase Activity (+/-)	Coagulase Activity (+/-)
01	-	*N/A*	*N/A*	*N/A*	*N/A*
02	-	*N/A*	*N/A*	*N/A*	*N/A*
03	-	*N/A*	*N/A*	*N/A*	*N/A*
04	+ + +	+ +	-	+ +	+ +
05	+ + +	+ +	-	+ +	+ +
11	+ + +	+ +	-	+ +	+ +
12	-	*N/A*	*N/A*	*N/A*	*N/A*
13	-	*N/A*	*N/A*	*N/A*	*N/A*
14	+ + +	+ +	-	+ +	+ +
15	-	*N/A*	*N/A*	*N/A*	*N/A*
21	-	*N/A*	*N/A*	*N/A*	*N/A*
22	-	*N/A*	*N/A*	*N/A*	*N/A*
23	+ + +	+ +	-	+ +	+ +
24	-	*N/A*	*N/A*	*N/A*	*N/A*
25	-	*N/A*	*N/A*	*N/A*	*N/A*
31	-	*N/A*	*N/A*	*N/A*	*N/A*
32	-	*N/A*	*N/A*	*N/A*	*N/A*
33	-	*N/A*	*N/A*	*N/A*	*N/A*
34	-	*N/A*	*N/A*	*N/A*	*N/A*
35	-	*N/A*	*N/A*	*N/A*	*N/A*
41	-	*N/A*	*N/A*	*N/A*	*N/A*
42	-	*N/A*	*N/A*	*N/A*	*N/A*
43	-	*N/A*	*N/A*	*N/A*	*N/A*
44	-	*N/A*	*N/A*	*N/A*	*N/A*
45	+ + +	+ +	-	+ +	+ +

Table 4: Results of Biochemical Tests for all environmental control samples, with *N/A* indicating that the test was not performed with the corresponding sample and multiple symbols indicating the results for all replicate tests done

Sample Number	Mannitol Fermentation (+/-)	DNase Activity (+/-)	Oxidase Activity (+/-)	Catalase Activity (+/-)	Coagulase Activity (+/-)
100	-	N/A	N/A	N/A	N/A
101	-	N/A	N/A	N/A	N/A
102	-	N/A	N/A	N/A	N/A
103	+++	+ +	-	+ +	+
104	-	N/A	N/A	N/A	N/A
105	-	N/A	N/A	N/A	N/A
106	+++	+ +	-	+ +	+
107	-	N/A	N/A	N/A	N/A
108	-	N/A	N/A	N/A	N/A
109	-	N/A	N/A	N/A	N/A
110	-	N/A	N/A	N/A	N/A
111	-	N/A	N/A	N/A	N/A
112	+++	+ +	-	+ +	+
113	-	N/A	N/A	N/A	N/A
114	+++	+ +	+	+ +	-
115	+++	+ +	-	+ +	+

Table 5: Planned primer sequences

Gene Name and Primer Direction	Primer Sequence
orfX forward	GAGAAATATTGGAAGCAAGCC
orfX reverse	CGCATAATCTTAAATGCTCTG
mecA forward	CTCATATAGCTCATCATACACTTTACC
mecA reverse	CACTTATTTTAATAGTTGTAGTTGTCGG
ccrC forward	CAGTAATGTCAAGATGTCGATGAATGC
ccrC reverse	CCGTCGACATACCATATTATTGCC
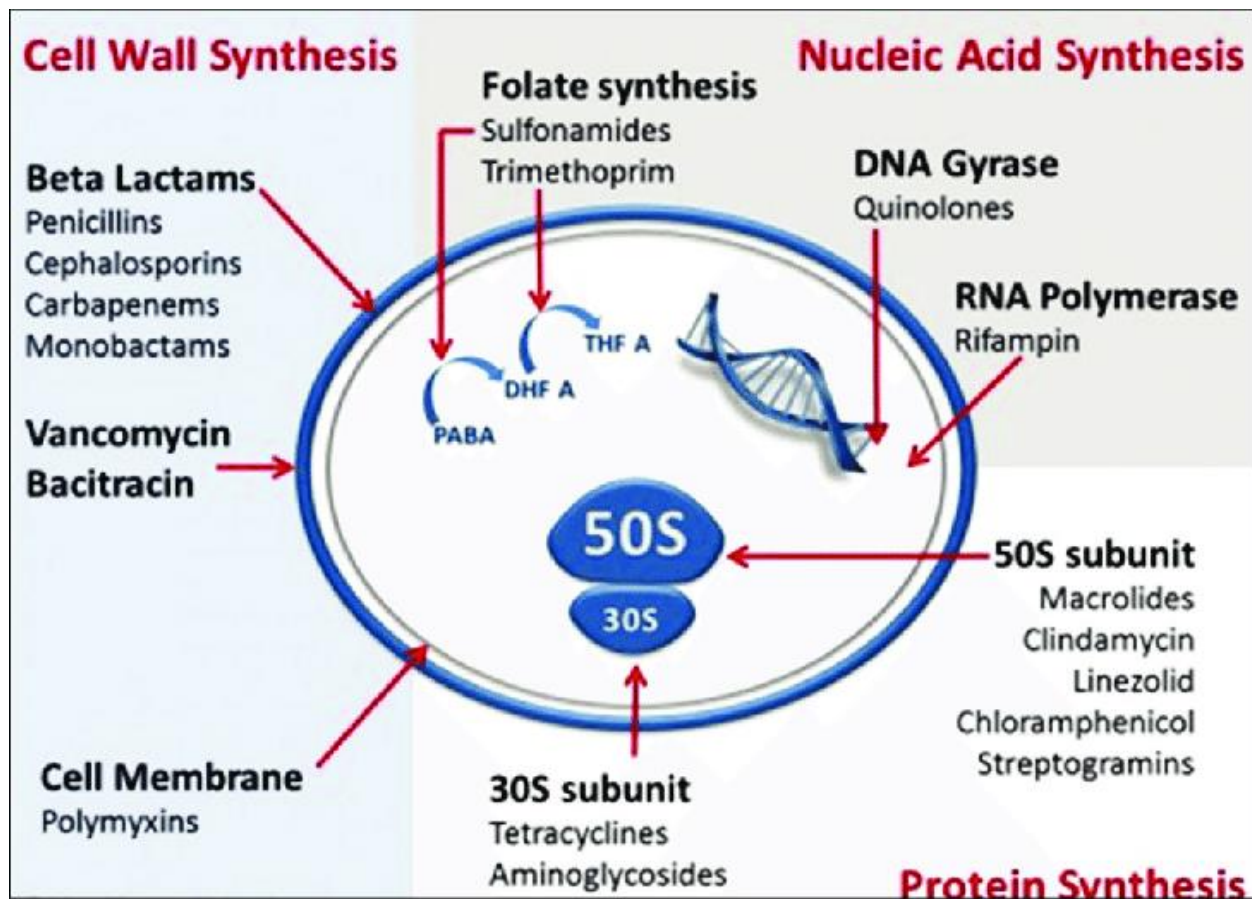
Figure 1: Mechanism of action of antibiotics (Kapoor, Saigal, & Elongavan, 2017)
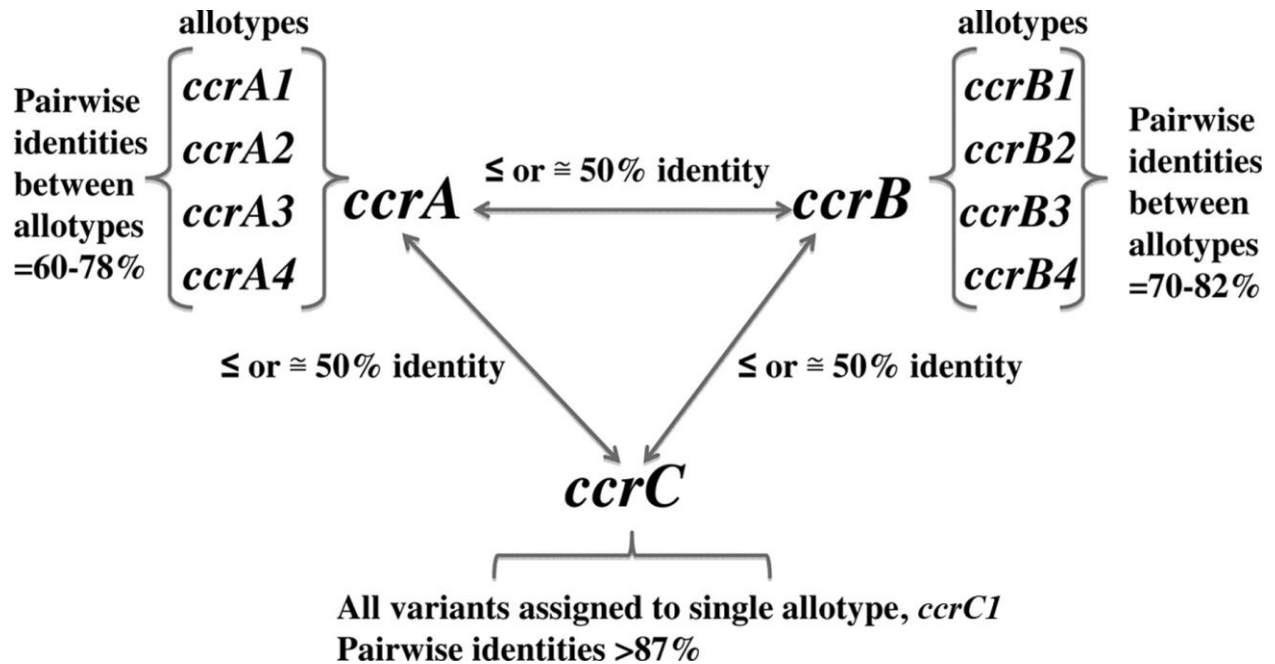
Figure 2: Relationships and naming conventions for *ccr* genes. Pairwise identity percentages can be thought of as percentages of relation, with the higher percentage representing a closer relationship. (International Working Group on the Classification of Stphylococcal Cassette Chromosome Elements, 2009)
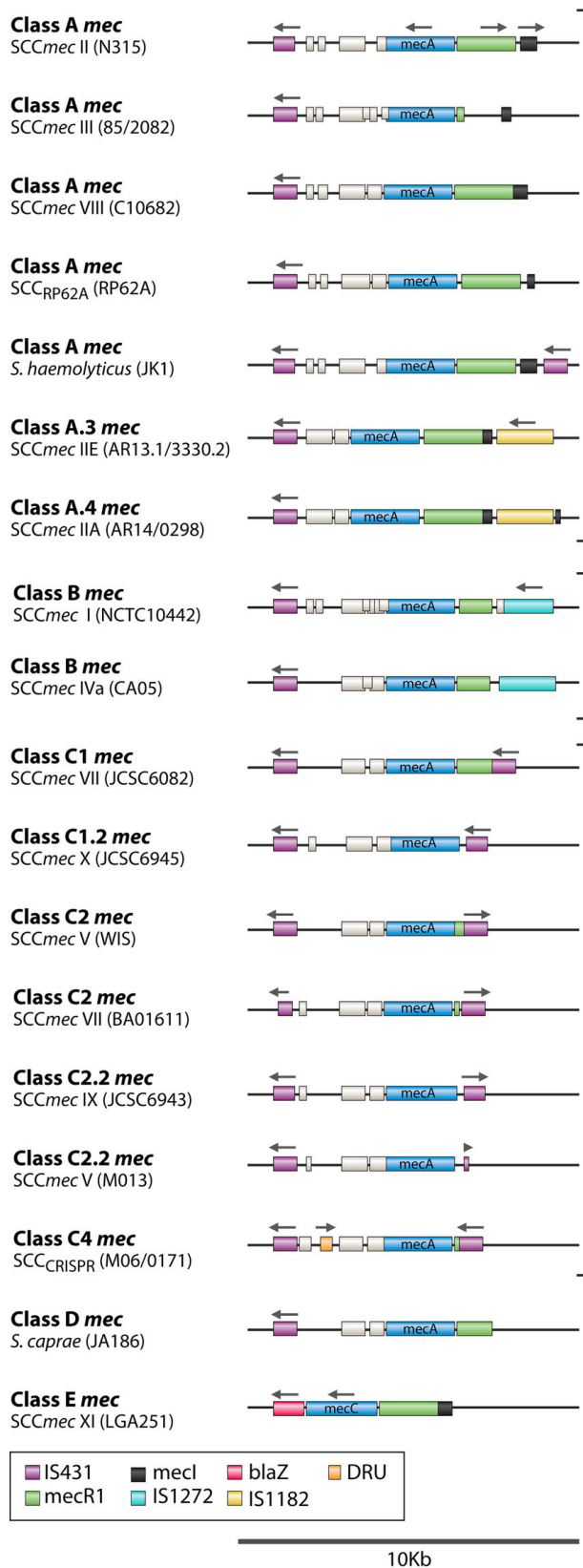
Figure 3: *mec* gene complex variants. (Lakhundi & Zhang, 2018)

Figures of Experimental Results
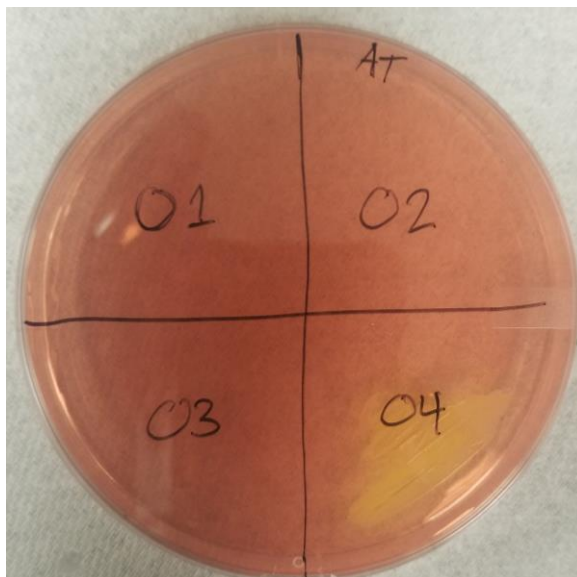
Figure 4: Initial MSA plate, including samples 01, 02, 03, and 04 (from top left to bottom right, respectively)
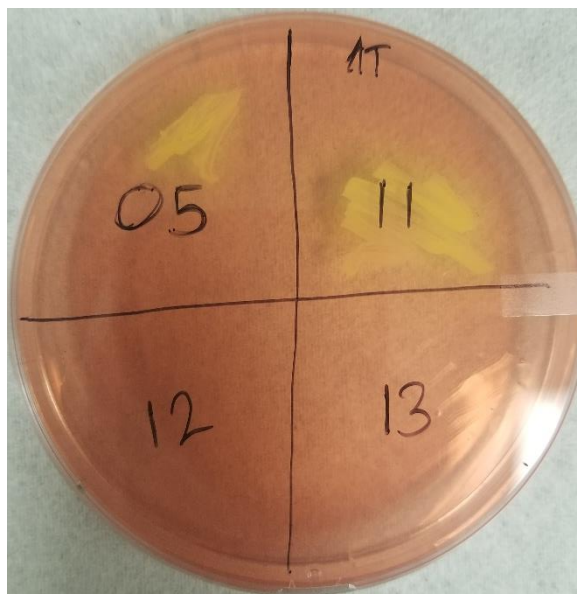
Figure 5: Initial MSA Plate, including samples 05, 11, 12, and 13 (from top left to bottom right, respectively)
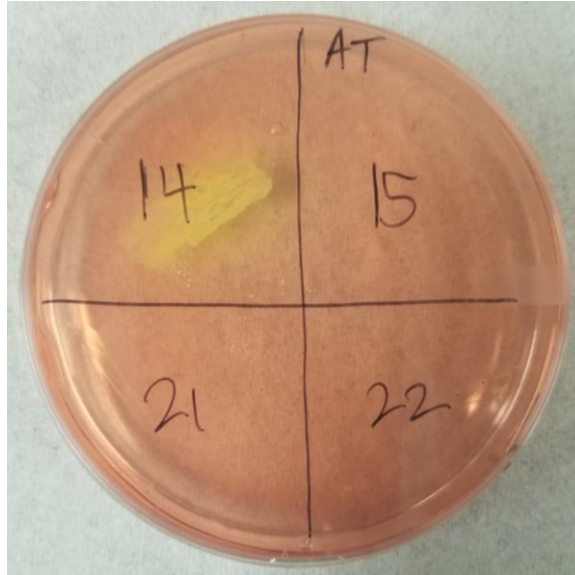
Figure 6: Initial MSA Plate, including samples 14, 15, 21, and 22 (from top left to bottom right, respectively)
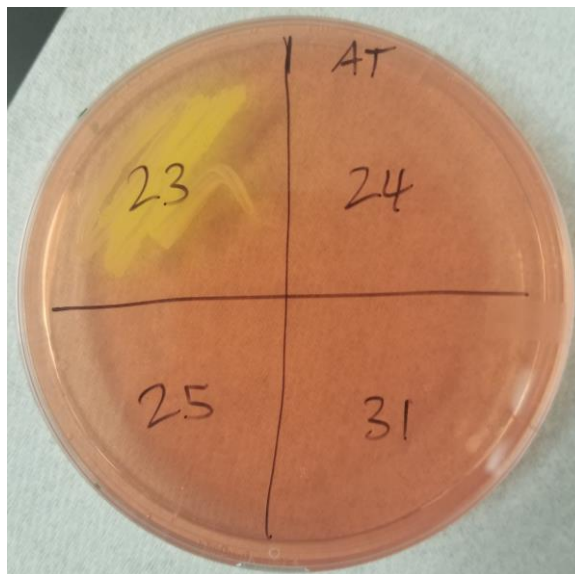
Figure 7: Initial MSA Plate, including samples 23, 24, 25, and 31 (from top left to bottom right, respectively)

Figure 8: Initial MSA Plate, including samples 32, 33, 34, and 35 (from top left to bottom right, respectively)
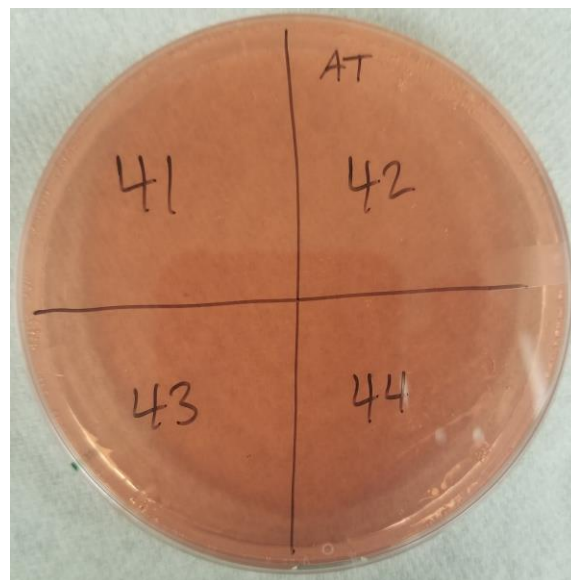
Figure 9: Initial MSA Plate, including samples 41, 42, 43, and 44 (from top left to bottom right, respectively)
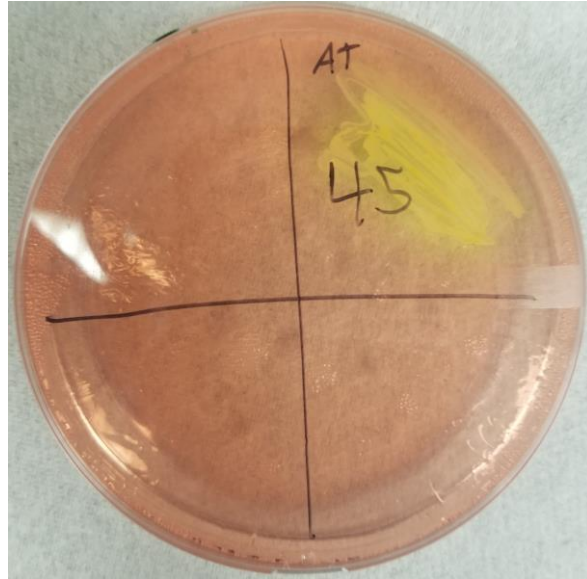
Figure 10: Initial MSA Plate, including sample 45
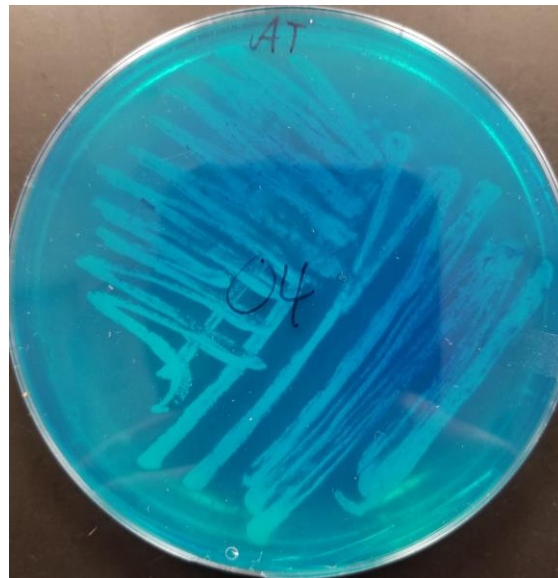
Figure 11: DNase Plate of Sample 04 after incubation with pH indicator solution
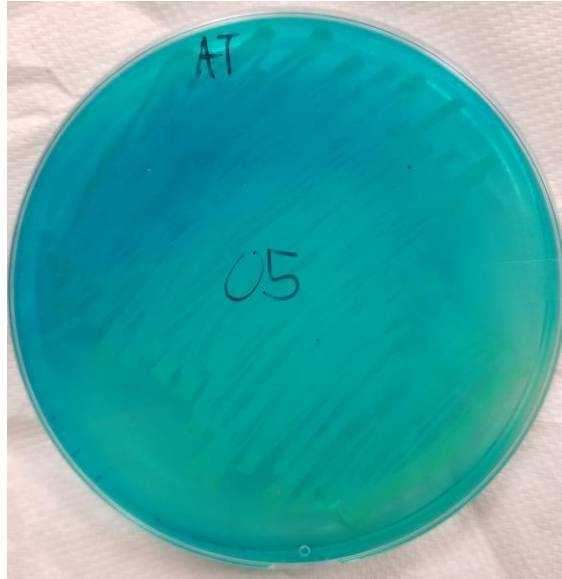
Figure 12: DNase Plate of Sample 05 after incubation with pH indicator solution
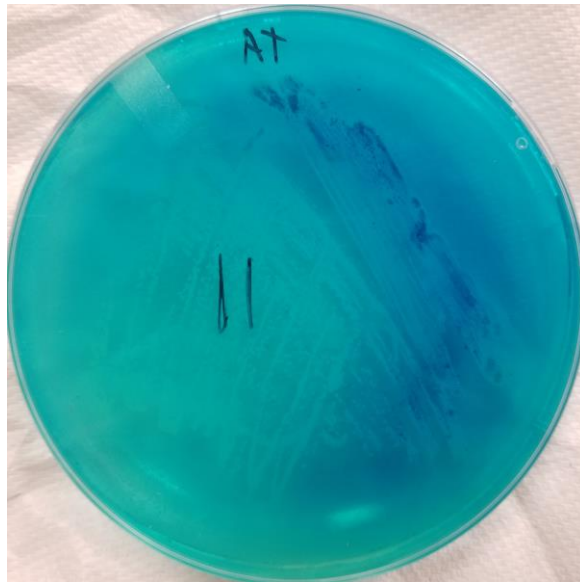
Figure 13: DNase Plate of Sample 11 after incubation with pH indicator solution
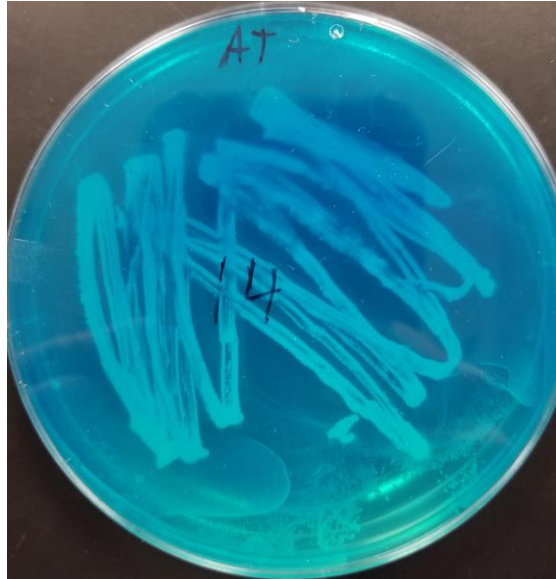
Figure 14: DNase Plate of Sample 14 after incubation with pH indicator solution
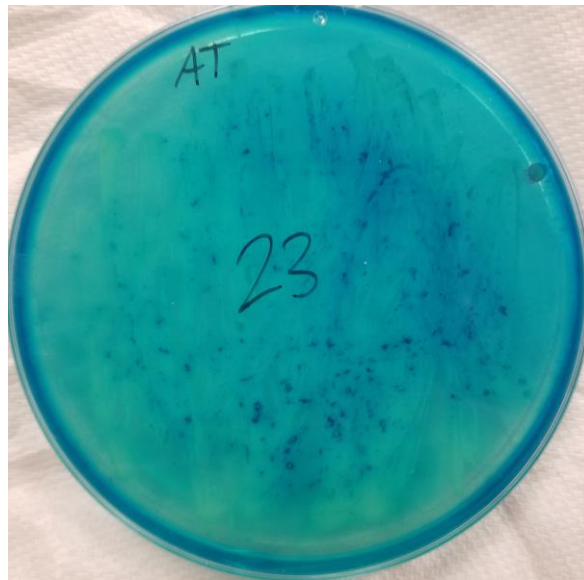
Figure 15: DNase Plate of Sample 23 after incubation with pH indicator solution
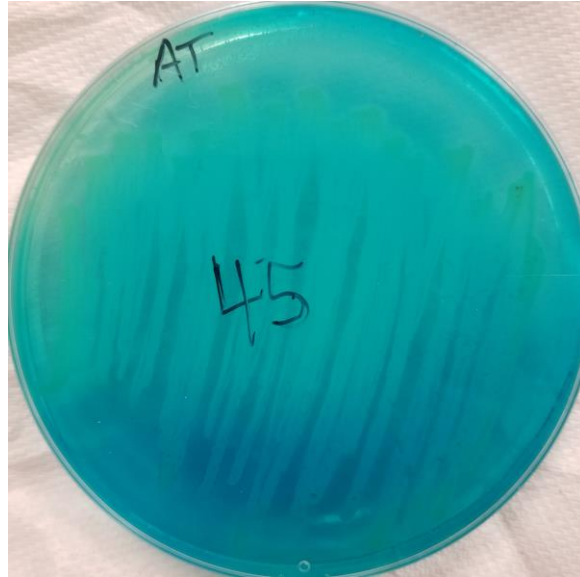
Figure 16: DNase Plate of Sample 45 after incubation with pH indicator solution
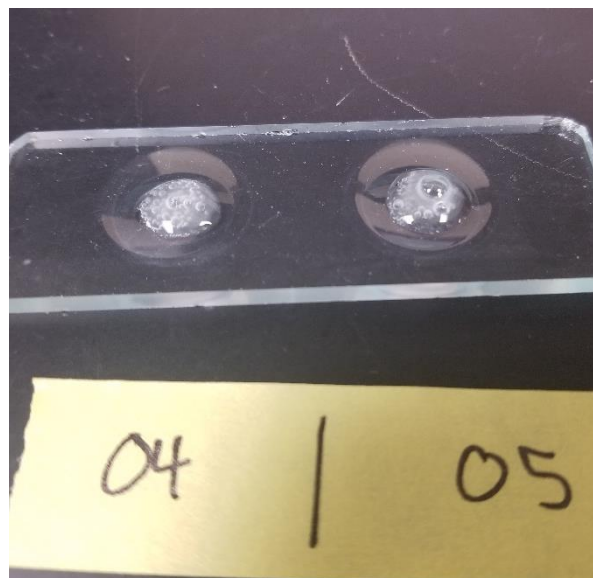
Figure 17: Catalase test results for Samples 04 and 05
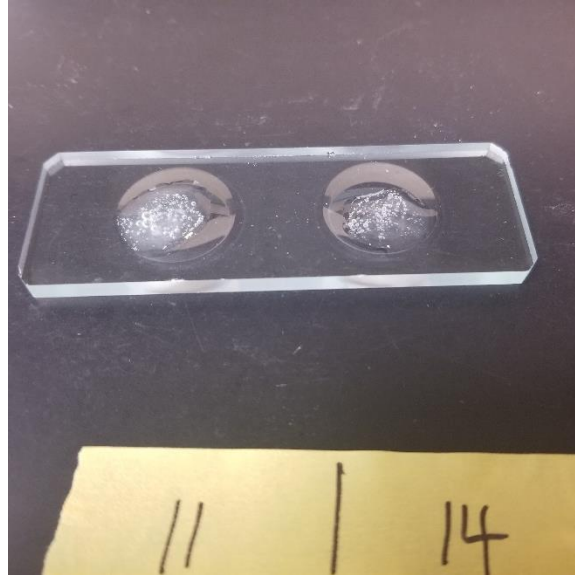
Figure 18: Catalase test results for Samples 11 and 14
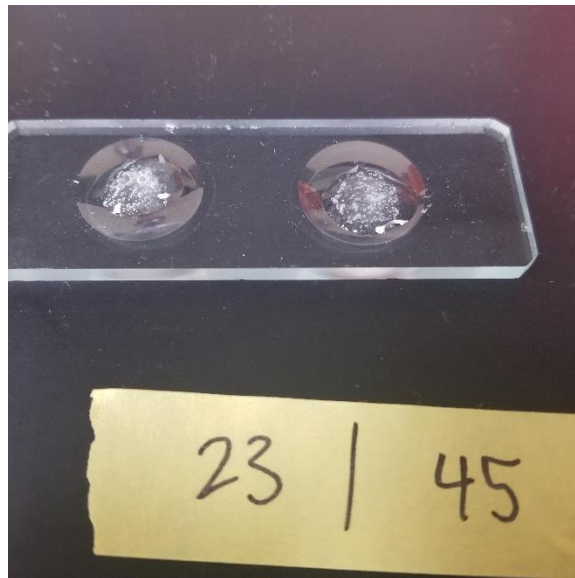
Figure 19: Catalase test results for Samples 23 and 45
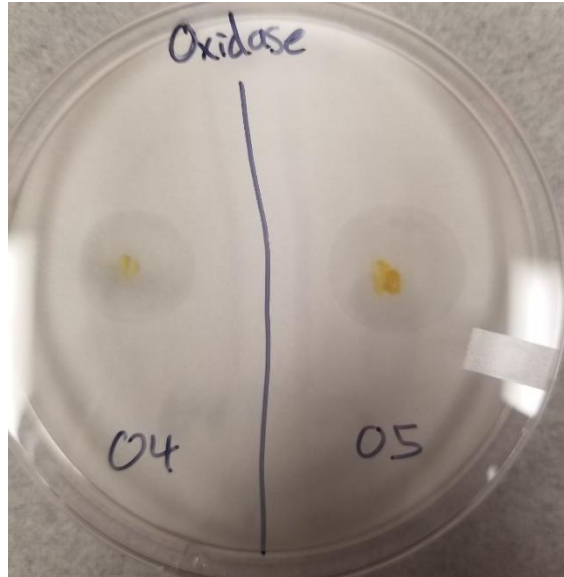
Figure 20: Oxidase test results for Samples 04 and 05
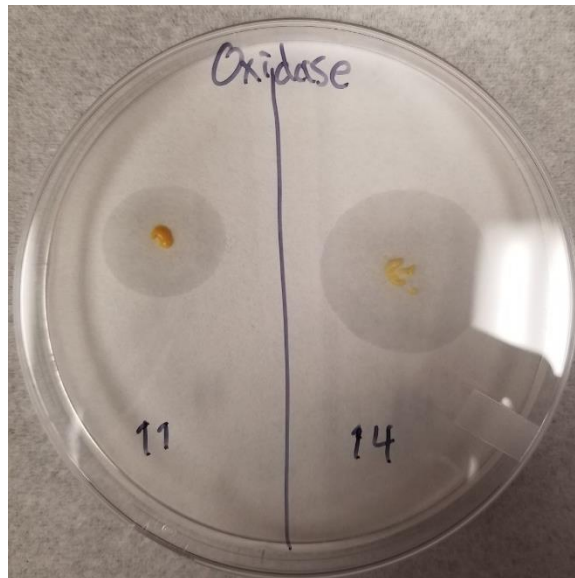
Figure 21: Oxidase test results for Samples 11 and 14
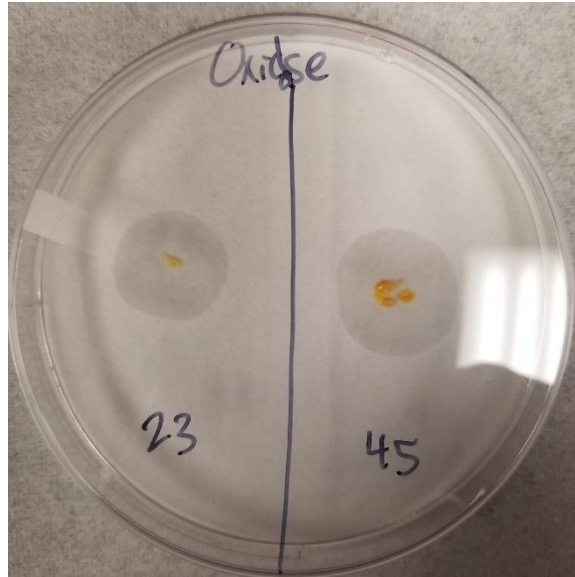
Figure 22: Oxidase test results for Samples 23 and 45
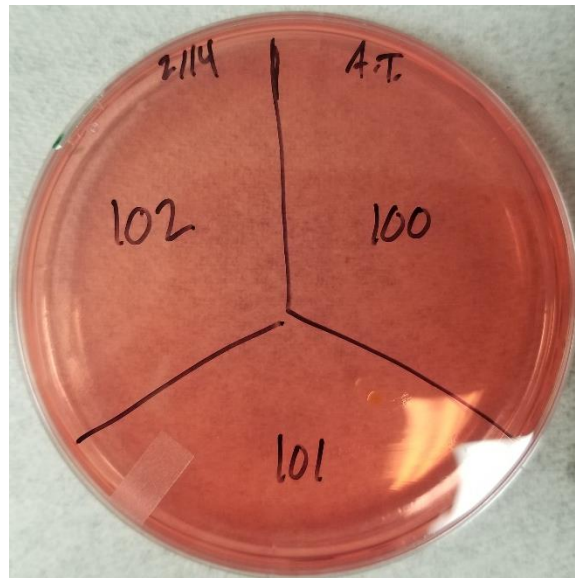
Figure 23: Initial MSA Plate, including samples 100, 101, and 102
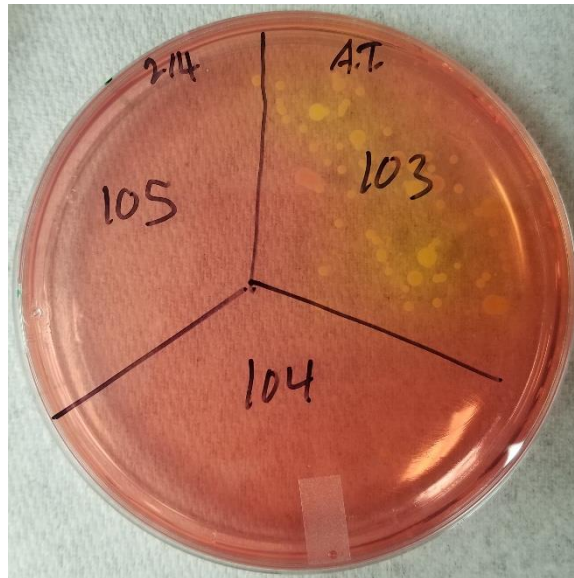
Figure 24: Initial MSA Plate, including samples 103, 104, and 105
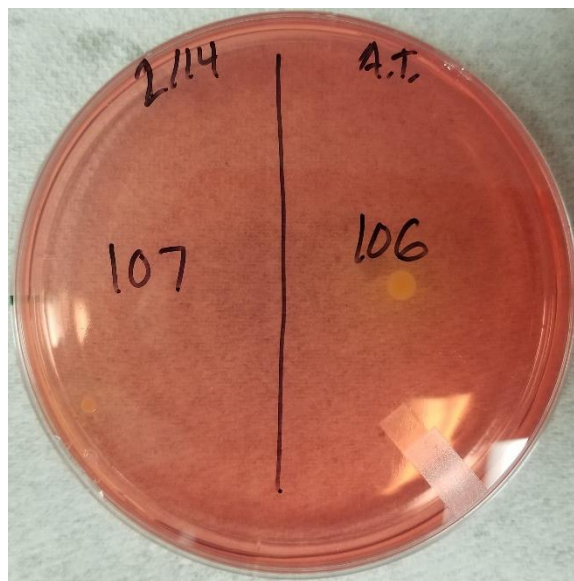
Figure 25: Initial MSA Plate, including samples 106 and 107
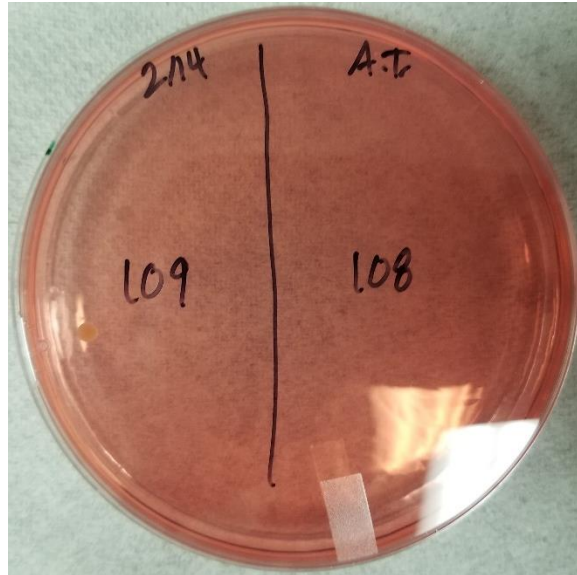
Figure 26: Initial MSA Plate, including samples 108 and 109

Figure 27: Initial MSA Plate, including samples 110, 111, and 112
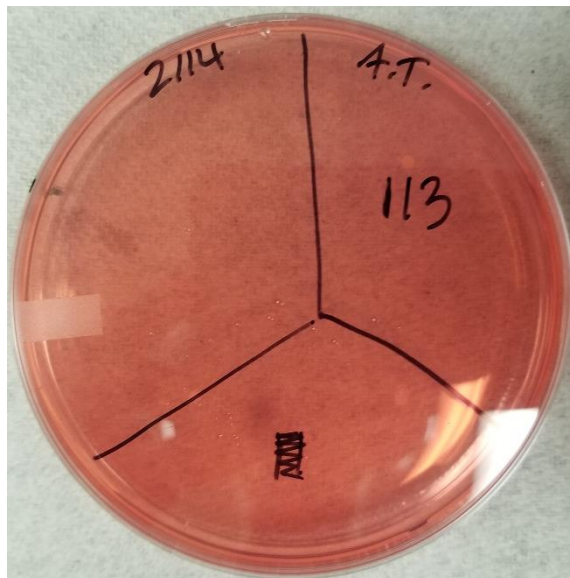
Figure 28: Initial MSA Plate, including sample 113
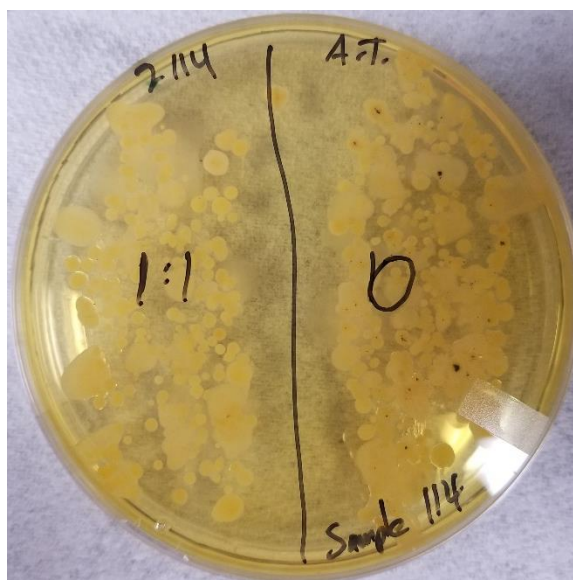
Figure 29: Initial MSA Plate, including undiluted and 1:1 dilution of Sample 114
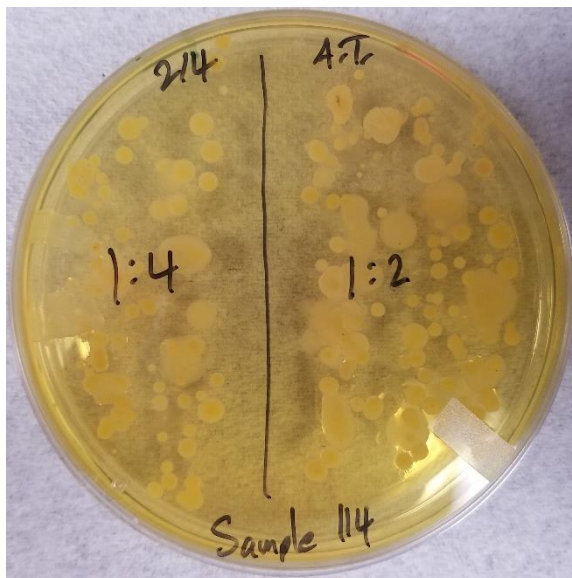
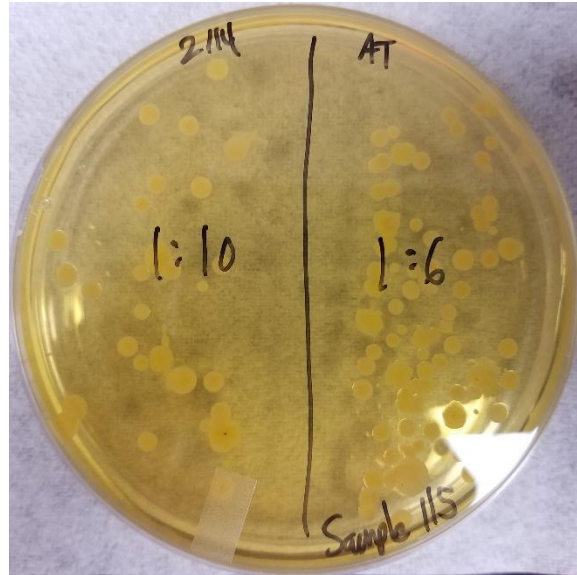Figure 30: Initial MSA Plate, including 1:2 and 1:4 dilutions of Sample 114
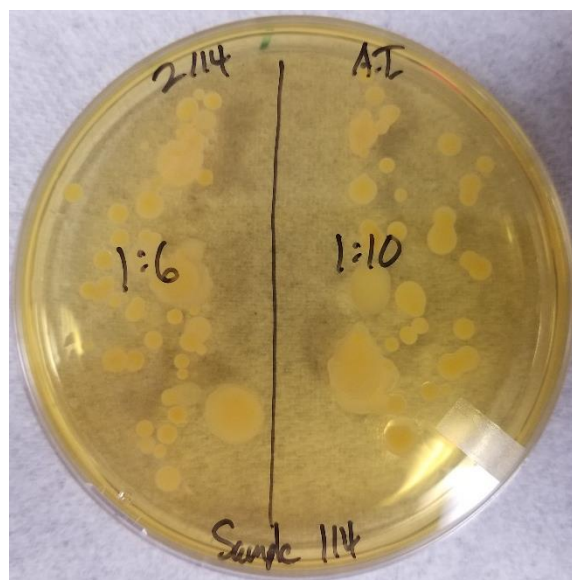
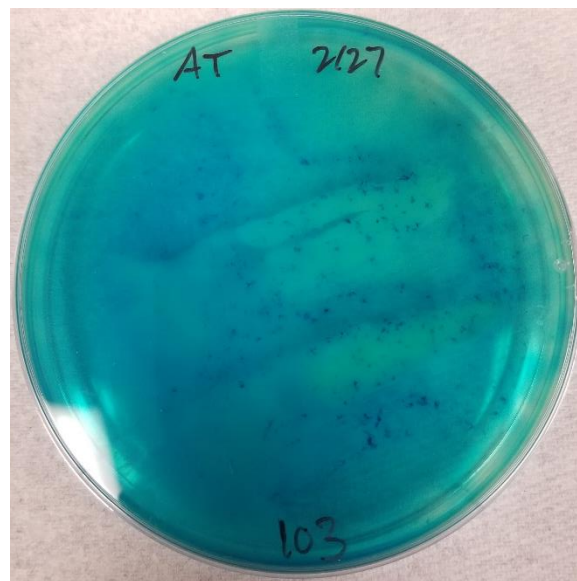Figure 31: Initial MSA Plate, including 1:6 and 1:10 dilutions of Sample 114
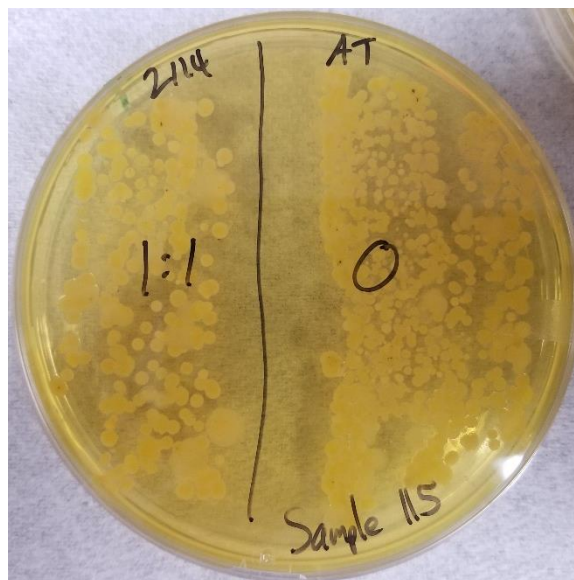
Figure 32: Initial MSA Plate, including undiluted and 1:1 dilution of Sample 115
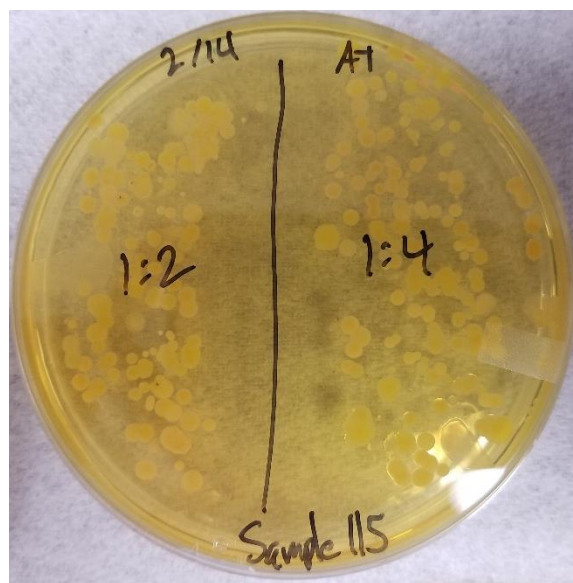
Figure 33: Initial MSA Plate, including 1:2 and 1:4 dilutions of Sample 115

Figure 34: Initial MSA Plate, including 1:6 and 1:10 dilutions of Sample 115

Figure 35: DNase Plate of Sample 103 after incubation with pH indicator solution
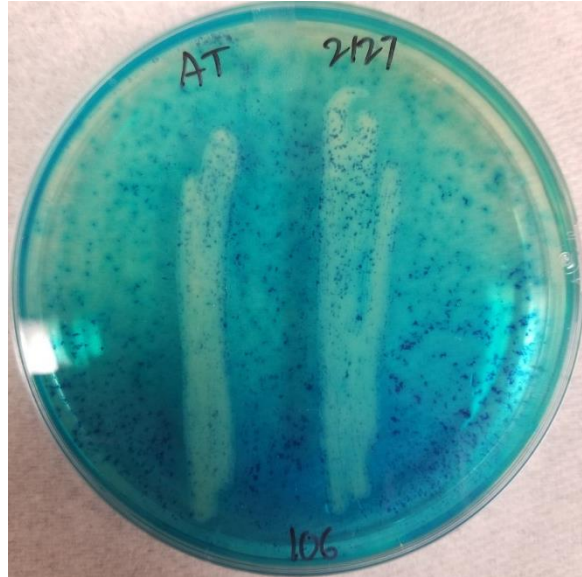
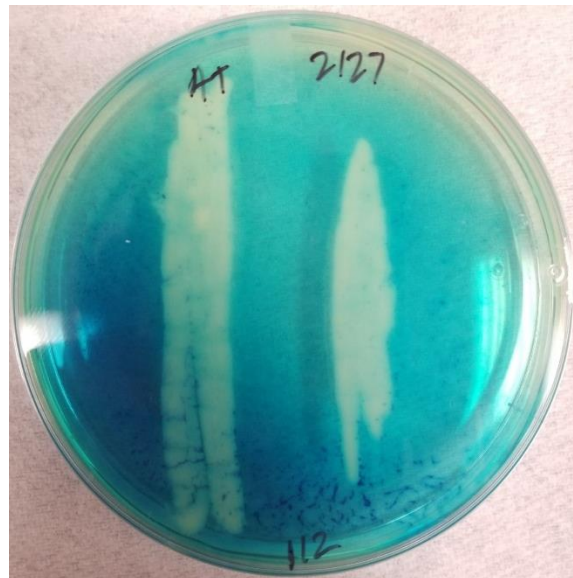Figure 36: DNase Plate of Sample 106 after incubation with pH indicator solution

Figure 37: DNase Plate of Sample 112 after incubation with pH indicator solution
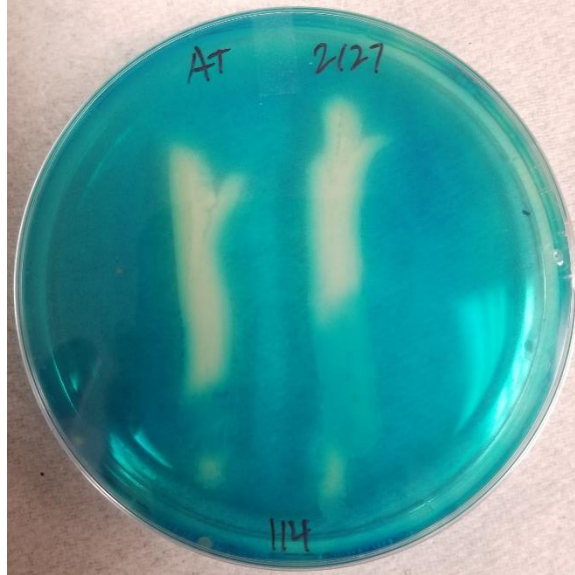
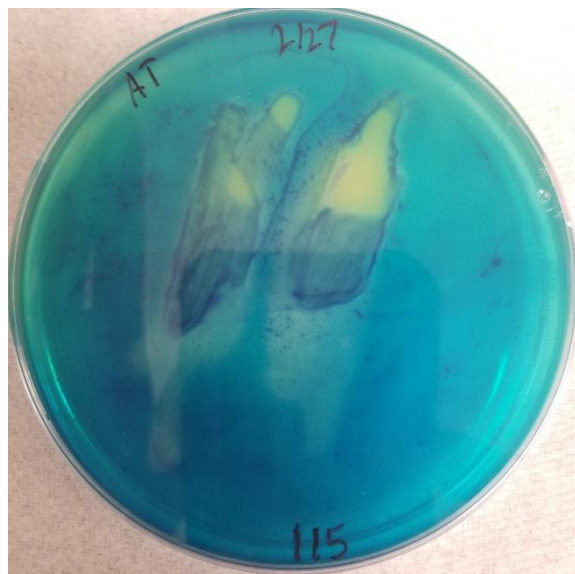Figure 38: DNase Plate of Sample 114 after incubation with pH indicator solution

Figure 39: DNase Plate of Sample 115 after incubation with pH indicator solution
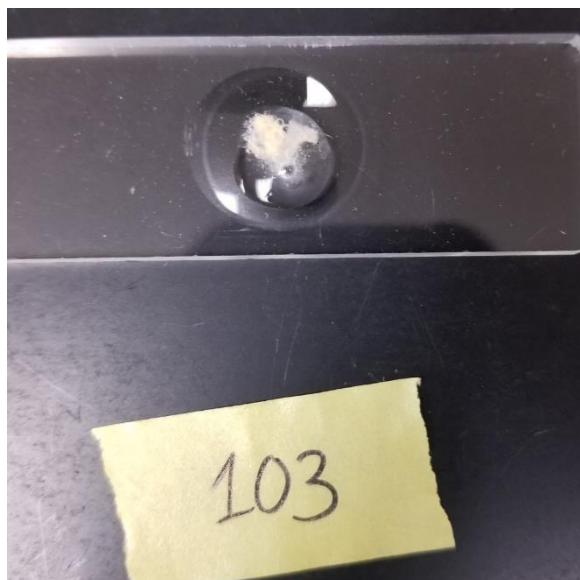
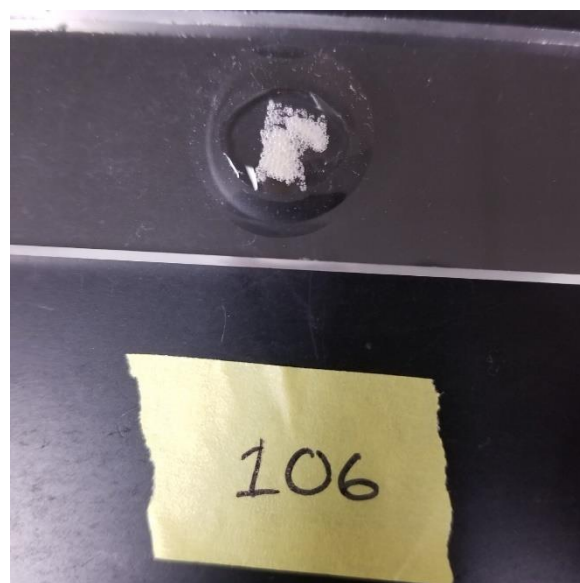Figure 40: Catalase Test results for Sample 103

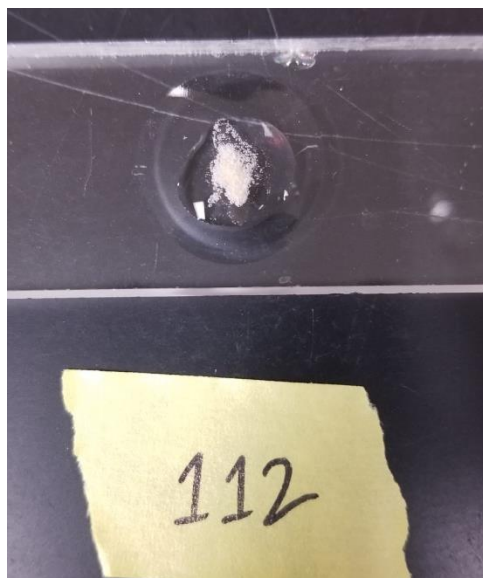Figure 41: Catalase Test results for Sample 106

Figure 42: Catalase Test results for Sample 112
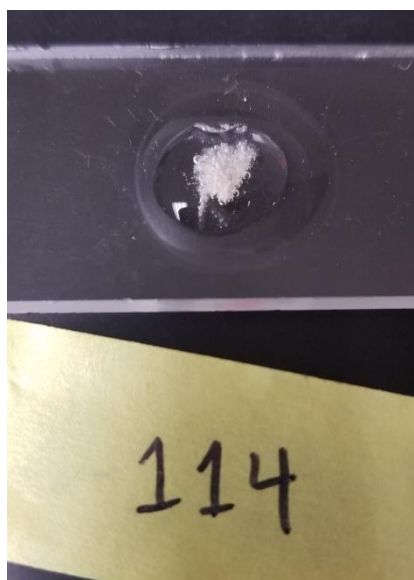

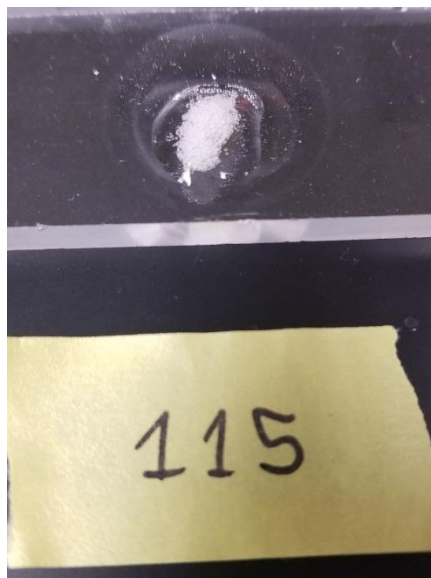Figure 43: Catalase Test results for Sample 114

Figure 44: Catalase Test results for Sample 115

Figure 45: Oxidase Test results for Sample 103

Figure 46: Oxidase Test results for Sample 106

Figure 47: Oxidase Test results for Sample 112
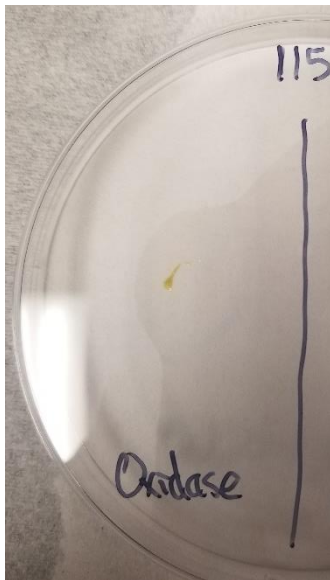
Figure 48: Oxidase Test results for Sample 114

Figure 49: Oxidase Test results for Sample 115

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