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Molecular monitoring of Methicillin-Resistant Staphylococcus aureus (MRSA) in a hospital setting

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Molecular Monitoring of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in a Hospital Setting

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"Tell me and I will forget, teach me and I may remember, ***involve me and I will learn****."*

- Benjamin Franklin

Acknowledgements

I would like to acknowledge Dr. David K. Giles for his guidance and support in my research the past two years. He has continuously worked to help me understand concepts and methodologies, shed light onto new opportunities, and to challenge myself. This project could not have been done without him. I would also like to acknowledge Colin M. Smith for assisting with lab work. Special thanks to Dr. Margaret J Kovach for providing access to equipment for PCR and gel documentation. A big thank you is owed to the Collaborative Research Initiative for Sponsored Programs (CRISP) grant, the UTC Honors College, and Provost Student Research Award for supporting this project.

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I dedicate this thesis to my family, Morgan Hardee, and Emmie. Their love, support, and encouragement in all that I do means more to me than they will ever know.

ABSTRACT

Methicillin resistant *Staphylococcus aureus* (MRSA) is a potentially pathogenic bacterium that poses a serious risk in healthcare settings. MRSA can be characterized by a genetic element, known as the staphylococcal cassette

chromosome, which harbors the gene responsible for methicillin resistance, *mecA.* MRSA can be classified into two categories: community acquired (CA) and hospital acquired (HA). *S. aureus* strains represent a major health concern due to their prevalence in healthcare facilities and their rapidly evolving antibiotic resistance. The current study investigated the association between MRSA isolates obtained from patients and from the intensive care units in a local hospital. Among the bacteria isolated from the neonatal and pediatric intensive care units were *Staphylococcus aureus, Staphylococcus epidermidis*, *Escherichia coli,* and members of the *Micrococcus* and *Bacillus* genera. MRSA isolates were confirmed by a combination of mannitol salt agar, CHROM agar, and antibiotic disc diffusion tests. Genomic DNA was extracted from the MRSA isolates by multiplex PCR to differentiate between CA and HA. We utilized multiple genomic markers to identify the *mecA* gene, differentiate the types of MRSA, and observe if specific toxins were present in twenty-five patient samples and eight environmental samples. In patient samples type II (HA), type III (HA), and type IVd (CA) were confirmed. In environmental samples type III (HA), type IVa (CA), and type V (CA) were confirmed. Both patient and environmental samples expressed the *mecA* gene indicative of MRSA. The only correlative genomic marker between patient and environmental samples was the type III and *mecA* gene; however, several isolates possessed *mecA* but did not match any of the types tested. Ongoing research involves the examination of over fifty more MRSA isolates, allowing further molecular characterization and determination of MRSA exchange in a healthcare setting.

Chapter 1

Introduction and Background

1.1 Multi-Drug Resistant Bacteria

Antimicrobials were developed by humans to reduce the impact of disease-causing microbes, with the most common type of antimicrobials being antibiotics. Most antibiotics target bacteria.[1] Penicillin, the first commercialized antibiotic, was discovered in 1928 by Alexander Fleming and began to be distributed to the general public in 1945.[2] The introduction of Penicillin was extremely useful for fighting surgical and wound infections with some referring to it as the "miracle drug".[3] Without the use of much needed antibiotics infected patients could experience increased recovery time, increased medical expenses, limb removal due to tissue necrosis, or even death. Unfortunately, over time certain bacteria developed strains that exhibited resistance to penicillin, creating a need for different antibiotics.[4] Throughout the following years new antibiotics were introduced including, but not limited to, tetracycline, erythromycin, methicillin, gentamicin, and vancomycin.[1]

Antibiotic	Year Antibiotic Introduced	Year Antibiotic Resistance Identified
Penicillin	1943	1965
Tetracycline	1950	1959
Erythromycin	1953	1968
Methicillin	1960	1962
Gentamicin	1967	1979
Vancomycin	1972	1988
Levofloxacin	1996	1996

Table 1.1 A timeline showing the evolution of antibiotic resistance in relation to antibiotic introduction.[5]

Antibiotic resistance occurs when bacteria are able to resist the effect of drugs- therefore the bacteria are not killed and their growth is not inhibited.[1] Throughout the 21st century antibiotic resistance has emerged to the forefront of public healthcare concerns.[6] In 2013, according to the Center for Disease Control and Prevention, a minimum of 2 million people in the United States experienced serious infections due to bacteria resistant to at least one antibiotic used to treat those infections, and at least 23,000 people died directly due to antibiotic-resistant

infections.[7] In Europe approximately 400,000 people were infected with multidrug-resistant bacteria leading to about 25,000 deaths in 2007.[8] The growing issue of drug resistant bacteria is a global concern that carries many implications and is a very complex, multifactorial issue.[6] There are various ways bacteria become resistant to antibiotics. It is possible for bacteria to "neutralize" an antibiotic by changing it in a way that essentially makes it harmless. Another way occurs when bacteria change their outer structure preventing the antibiotic from attaching to the bacteria it is programmed to kill.[9] Overuse and misuse of antibiotics can contribute to the development of antibiotic-resistant bacteria. A well-known group of drug resistant bacteria are the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species).[10] They are able to "escape" the actions of antibiotics and are the leading cause of Healthcare Associated Infections (HAIs) throughout the world.[10,11]

1.2 *Staphylococcus aureus*

Staphylococcus aureus was discovered in the 1880s by surgeon Sir Alexander Ogston.[12] It was found to be a Gram-positive bacterium that is responsible for causing a wide range of infections and diseases, varying from minor skin infections to post-operative wound infections, necrotizing pneumonia, and bacteremia.[13,14] *S. aureus* can express resistance to many antibiotics.[15,16] Early on the mortality rate for patients infected with *S. aureus* was around 80%; however, in

the mid 1900s *S. aureus* infections began to be treated with penicillin.[17] This helped to lower the mortality rate for infected patients. Resistance to penicillin emerged in 1942 due to the acquisition of a plasmid that encoded a penicillin-hydrolyzing enzyme (penicillinase) and in only 18 years 80% of *S. aureus* strains were unaffected by the drug.[17,18,19] However, new generations of modified beta lactam antibiotics were briefly effective against *S. aureus* until 1961.[18,20,21,22] In 1961, just two years after methicillin was introduced, which is a semisynthetic form of penicillin, *S. aureus* strains emerged that were resistant to both methicillin and beta lactam antibiotics in general due to their acquisition of the *mecA* gene.[17,18] *S. aureus* strains have even developed resistance to other antibiotics like vancomycin if they harbor the *vanA* gene, making this pathogen one of the most difficult to treat, particularly in clinical settings where it rapidly evolves and is easily spread.[18,23,24]

1.3 The *mecA* gene

The existence of methicillin resistant *S. aureus* (MRSA) has placed a remarkable burden on the public health care system, where accurate molecular characterization is crucial for infection control and surveillance of the bacteria.[25] MRSA can be characterized by a genetic element, known as staphylococcal cassette chromosome *mec* (SCC*mec*) that is indicated by roman numerals I to XIII.[25,26,27] The

SCC*mec* harbors the gene responsible for methicillin resistance, *mecA,* and the *ccr* gene complex responsible for genetic mobility.[26] The *mecA* gene encodes a penicillin-binding protein (PBP) 2A or PBP2'. Since β-lactam antibiotics (like penicillin, or methicillin which is a semisynthetic derivative of penicillin) cannot bind to PBP2', synthesis of peptidoglycan layer and cell wall synthesis are able to continue.[26,27,28]

In 2011 it was reported that MRSA was capable of encoding a divergent *mecA* gene. This homologue known as *mecC,* previously known as $mecA_{LGA251}$, has the potential to be misdiagnosed as methicillin sensitive *S. aureus*, complicating patient management as well as MRSA surveillance.[28]

1.4 Community and Hospital Acquired

During the late 1900's two different populations of MRSA began to emerge, known as hospital acquired (HA) and community acquired (CA).[29] HA-MRSA is usually associated with people who have had frequent or recent contact with healthcare facilities within the past year, or have recently undergone an invasive medical procedure.[29,30,31] HA-MRSA is identified as types I, II, and III. It is a serious infection resistant to multiple drugs and infections occur at sites including the blood, skin, and lungs.[29,32] CA-MRSA is associated with people who have not been in the healthcare facility or had a medical procedure within the past year. CA-MRSA typically consists of skin and soft tissue infections and is identified as types IV and

V MRSA.[33] While SCC*mecA* typing helps us to differentiate CA vs. HA, determining the true origin of each MRSA type is problematic. In recent years, researchers have been able to identify HA-MRSA in the community and vice versa blurring the line between HA and CA.[34] The HA-MRSA could be evolving in healthcare facilities or could be brought into healthcare facilities by patients, visitors, etc.[34]

Chapter 2

Materials and Methodology

2.1 Sample Collections

Dr. Spratt and his student research assistants, including Colin Smith, worked with staff at Erlanger hospital to coordinate times to sample the NICU and PICU where they would swab various areas of the environment. These areas included stethoscopes, bed handles, bath basins, equipment drawers, computers, air ducts, floors, etc. Thermo Fisher Scientific sterile transport swabs with liquid Stuart's medium were used to collect these samples. All swabs were placed on ice immediately after being collected. Patient samples were provided directly from the medical technology lab at Erlanger. Other than the fact that these samples came from patients in the NICU, we are unsure of the process used by the medical technology lab to obtain and classify these samples. [35,36]

Figure 2.1. Sampling of Hospital Environment

2.2 Lab Processing

Dr. Spratt and his team processed environmental swabs collected at Erlanger in a lab at UTC within two hours of collection. In the lab these swabs were used to inoculate onto six different bacteria growth mediums, five classified as selective & differential, and one non-specific growth medium:

- CHROM MRSA agar- selective and differential for methicillin resistant *S. aureus (*MRSA)[37]
- Mannitol Salt Agar (MSA)- selective and differential for Staphylococci[38]
- Eosin Methylene Blue (EMB)- selective and differential for Gram negative enterics[39]
- Pseudomonas Isolation Agar (PsI)- selective and differential for *Pseudomonas*[40]
- MacConkey's Agar (MAC)- selective and differential for enterics[41]
- Tryptic Soy Agar (TSA)- non-specific, supports growth of many different species[42,43]

2.3 MRSA Characterization

Environmental isolates from CHROM, MSA, and TSA were then line inoculated onto MSA agar that indicates mannitol fermentation by turning agar from a deep red color to a bright yellow.[44]

Antibiotic disk diffusion tests were carried out on isolates showing positive for mannitol fermentation, to assess the strain's resistance to four common beta-lactam antibiotics: Penicillin, Amoxicillin, Oxacillin, and Vancomycin.[45] Confirmed MRSA isolates were passed on to Dr. Giles and I for further characterization.

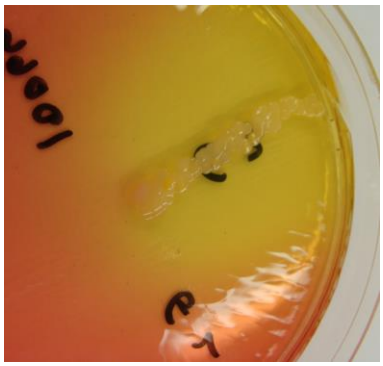


Figure 2.2. MSA testing for *S. aureus* characterization

2.4 Genomic Extraction

Upon receiving an 80% glycerol stock of confirmed MRSA isolates we grew overnight cultures to use for genomic extractions. Bacterial genetic DNA was then extracted using a Thermo Fisher Scientific kit and following the manufacturer's instructions. Afterwards, quality and quantity of genomic DNA extracted was assessed using a Nanodrop spectrophotometer.[46]

2.5 Multiplex Polymerase Chain Reaction

Multiplex PCR was utilized for staphylococcal cassette chromosome *mec* typing with primers designed for SCC*mec* types, SCC*mec* subtypes, toxins, and the *mecA* gene.[47,48,49,50,51] The PCR mixture included DNA, various primers, and master mix in a 25 µl final reaction (Figure 3) volume. Thermocycling condition were 94°C for 5 min, followed by 10 cycles of 94°C for 45 s, 65°C for 45 s, and 72°C for 90 s, followed by 25 cycles of 94°C for 45 s, 55°C for 45 s, and 72°C for 90 s, followed by 72°C for 10 min.[34]

Figure 2.3. PCR Samples

Primer	Oligonucleotide sequence	Amplicon Size (bp)
mecA	MecA147-F (5'- GTGAAGATATACCAAGTGATT-3')	147
	MecA147-R (5'-ATGCGCTATAGATTGAAAGGAT-3')	
SCC*mec* Type I	Type I-F (5'-GCTTTAAAGAGTGTCGTTACAGG-3')	613
	Type 1-R (5'-GTTCTCTCATAGTATGACGTCC-3')	
SCC*mec* Type II	Type II-F (5'-CGTTGAAGATGATGAAGCG-3')	398
	Type II-R (5'-CGAAATCAATGGTTAATGGACC-3')	
SCC*mec* Type III	Type III-F (5'-CCATATTGTGTACGATGCG-3')	280
	Type III-R (5'-CCTTAGTTGTCGTAACAGATCG-3')	
SCC*mec* Type IVa	Type IVa-F (5'-GCCTTATTCGAAGAAACCG-3')	776
	Type IVa-R (5'-CTACTCTTCTGAAAAGCGTCG-3')	
SCC*mec* Type IVb	Type IVb-F (5'-TCTGGAATTACTTCAGCTGC-3')	493
	Type IVb-R (5'-AAACAATATTGCTCTCCCTC-3')	
SCC*mec* Type IVc	Type IVc-F (5'-ACAATATTTGTATTATCGGAGAGC-3')	200
	Type IVc-R (5'-TTGGTATGAGGTATTGCTGG-3')	
SCC*mec* Type IVd	Type IVd-F (5'-CTCAAAATACGGACCCCAATACA-3')	881
	Type IVd-R (5'-TGCTCCAGTAATTGCTAAAG-3')	
SCC*mec* Type V	Type V-F (5'-GAACATTGTTACTTAAATGAGCG-3')	325
	Type V-R (5'-TGAAAGTTGTACCCTTGACACC-3')	
Panton-Valentine	Luk-PV-1 (5'-ATCATTAGGTAAAATGTCTGGACATGATCCA-3')	433
Leukocidin (PVL)	Luk-PV-2 (5'-GCATCAAGTGTATTGGATAGCAAAAGC-3')	
Toxic Shock	GTSSTR-1 (5'-ACCCCTGTTCCCTTATCATC-3')	326
Syndrome Toxin	GTSSTR-2 (5'-TTTTCAGTATTTGTAACGCC-3')	
mecA	mecI-F (5'-CCCTTTTTATACAATCTCGTT-3')	146
	mecI-R (5'-ATATCATCTGCAGAATGGG)	
ccrAB	ccrAB-β2 (5'-ATTGCCTTGATAATAGCCITCT-3')	
	ccrAB-α2 (5'-AACCTATATCATCAATCAGTACGT-3')	700
	ccrAB-α3 (5'-TAAAGGCATCAATGCACAAACACT-3')	1,000
	ccrAB-α4 (5'-AGCTCAAAAGCAAGCAATAGAAT-3')	1,600
ccrC	ccrC-F (5'-ATGAATTCAAAGAGCATGGC-3')	336
	ccrC-R (5'-GATTTAGAATTGTCGTGATTGC-3')	

Table 2.1 List of primers used in this study.[26,34]

2.6 Gel Electrophoresis

We made a 1.5% gel using the following procedure: put 0.6 g Agarose

powder into an Erlenmeyer flask, add 40 mL of TBE 1X, heat until the Agarose powder is dissolved, then add 2 µl of 10 mg/µl EtBr before pouring the mixture into the apparatus. After the gel in the apparatus has solidified, load the lanes with a 100 bp ladder and PCR samples and run it at 120 V.[52, 53, 54, 55, 56,57]

Chapter 3

Results

3.1 Nanodrop Spectrophotometer Results

Upon completing our genomic extractions we utilized a nanodrop spectrophotometer to analyze the concentration and purity of our DNA. We found lysostaphin is a more efficient method of lysing *S. aureus* and ultimately produces a higher yield of DNA. A 260/280 ratio of ~1.8 is generally considered "pure" for DNA. A 260/230 ratio of ~2.0-2.2 is generally considered "pure" for nucleic acid.

Patient Samples	**DNA Concentration (ng/µl)**	**260/280**	**260/230**
1	10.1	2.88	8.99
2	12.3	2.39	2.13
3	19.0	2.25	1.91
4	17.7	2.30	2.15
5	30.9	2.30	2.24
6	18.7	1.76	1.12
7	6.50	3.24	3.16
8	38.0	2.27	2.42

Table 3.1. Results from nanodrop spectrophotometer after genomic extractions were completed on patient samples 1-8.

Table 3.2. Results from nanodrop spectrophotometer after genomic extractions were completed on patient samples 9-15.

Patient Samples	DNA Concentration (ng/µl)	260/280	260/230
9	11.9	2.11	1.61
10	557.1	1.34	0.64
11	380.0	1.36	0.63
12	419.0	1.28	0.62
13	340.2	1.35	0.61
14	656.0	1.35	0.61
15	166.9	1.36	0.66

Patient Samples	DNA Concentration (ng/µl)	260/280	260/230
18	361.8	1.96	1.20
19	205.2	2.02	1.38
20	481.1	1.60	0.80
21	107.7	1.85	1.23
22	164.3	1.97	1.43
23	197.2	1.90	1.26
24	1.61	1.68	0.93
25	64.1	1.83	1.13
26	153.9	1.32	0.71

Table 3.3. Results from nanodrop spectrophotometer after genomic extractions were completed on patient samples 18-26

Environmental Samples	DNA Concentration (ng/µl)	260/280	260/230
1c	123.1	1.94	1.67
2c	340.7	2.00	1.19
3c	137.9	1.94	1.12
4c	53.0	2.00	1.26
5c	384.6	2.00	1.15
6c	289.3	2.02	1.23
7c	112.4	1.94	1.09
8c	60.6	2.00	1.33

Table 3.4. Results from nanodrop spectrophotometer after genomic extractions were completed on environmental samples 1c-8c.

Control Samples	DNA Concentration (ng/µl)	260/280	260/230
BAA-41	120.7	1.85	1.24
BAA-2094	93.5	1.68	0.91
33592	73.5	1.78	1.18

Table 3.5. Results from nanodrop spectrophotometer after genomic extractions were completed on control samples.

3.2 Multiplex PCR Characterization of Patient Samples

Initially observing patient samples 1-8, which were all labeled as MRSA by Erlanger hospital, 4 samples were not positive for the *mecA* gene that is indicative of MRSA (**Figure 3.6**), samples 2, 4, 6, and 8. We repeated the multiplex PCR run and gel with the same primers to confirm these 4 samples were negative for *mecA*. In this run only 2 samples, samples 4 and 8, were negative for the *mecA* gene (**Figure 3.7**). Diving into the literature for answers we found there is a divergent *mecA* gene known as *mec*$_{LGA251}$, also referred to as *mecC.* After finding this out, we purchased the appropriate primers and tested samples 1-8 for *mec*$_{LGA251}$, but samples 4 and 8 were negative for *mecA* and *mec*$_{LGA251}$ (**Figure 3.8**). Due to this we did not believe patient samples 4 and 8 were MRSA, but suspected they were MSSA.

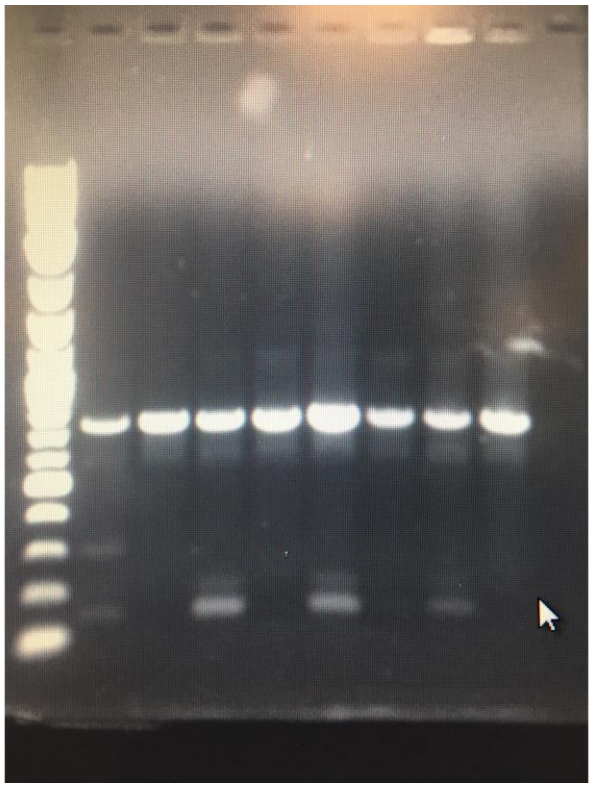


Figure 3.1. Gel of patient samples 1-8 from January 18th, 2017. Primers used are as follows: type I, II, III, IVa, IVb, IVc, IVd, V and *mecA*1417. The *mecA* gene is positive for samples 1, 3, 5, and 7. The *mecA* gene is negative for samples 2, 4, 6, and 8.

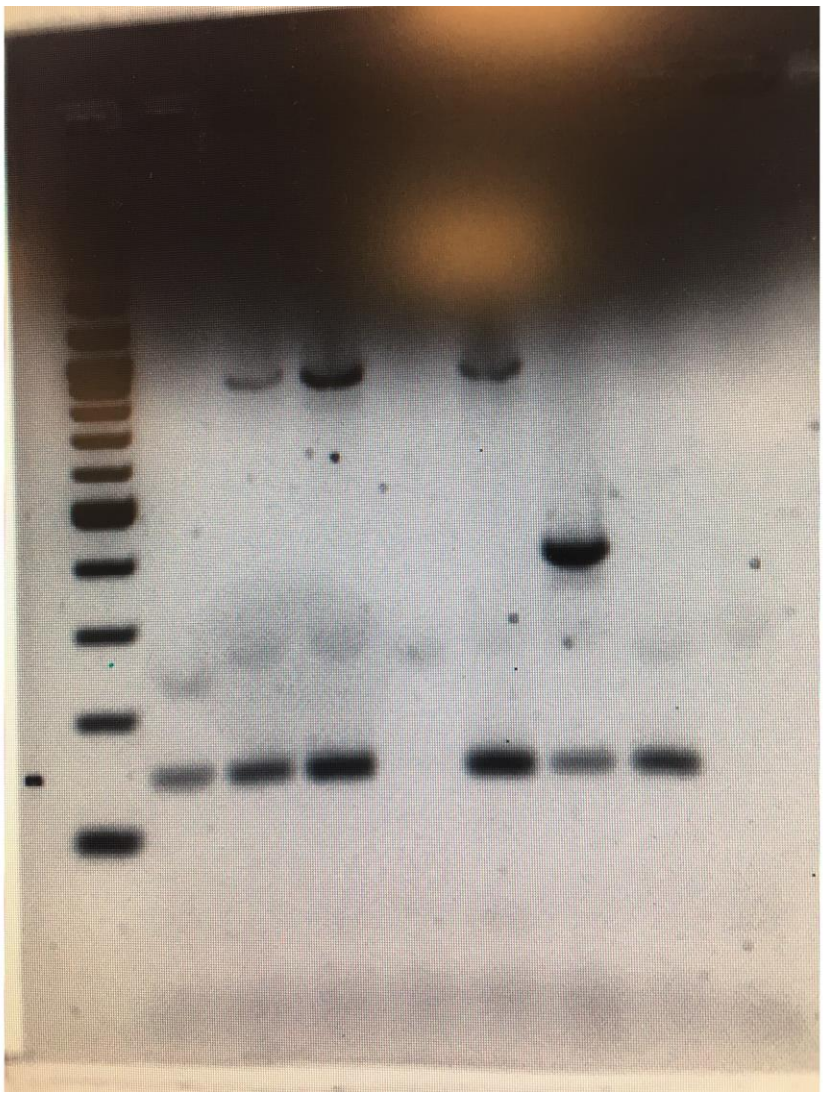


Figure 3.2. Gel of patient samples 1-8 from January 20th, 2017. Primers used are as follows: type I, II, III, IVa, IVb, IVc, IVd, V and *mecA*1417. The *mecA* gene is positive for samples 1, 2, 3, 5, 6, and 7. The *mecA* gene is negative for samples 4 and 8. In patient samples 2, 3, and 5 a strong band is seen at ~900 bp, indicating type IVd. In patient sample 6 there is a strong band at ~400 bp, indicating type II.

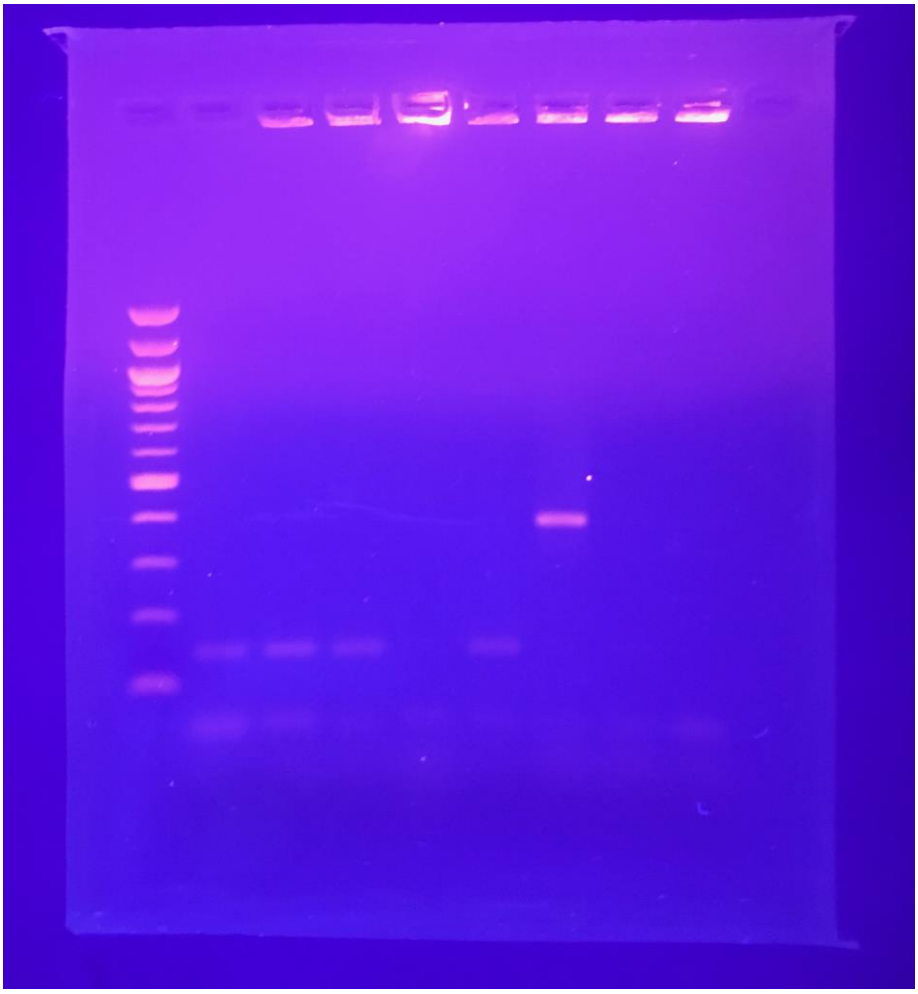


Figure 3.3. Gel of patient samples 1-8 from November 6th, 2017. Primers used are as follows: type I, II, III, IVa, IVb, IVc, IVd, V mecA1417, and *mec*$_{LGA251}$. The *mecA* gene is positive for samples 1, 2, 3, 5, 6, and 7. The *mecA* and *mec*$_{LGA251}$ gene is negative for patient samples 4 and 8. In patient sample 6 there is a strong band at ~400 bp, indicating type II.

As far as SCC*mec* types, throughout these 3 Multiplex PCR runs and gels we identified patient samples 2, 3, and 5 to be positive for type IVd and patient sample 6 to be positive for type II (**Figures 3.1, 3.2, 3.3**).

After characterizing patient samples 1-8, we moved on to samples 9-15. Samples 9-13 were labeled as MRSA and samples 14 and 15 were labeled MSSA by Erlanger hospital. In our first gel our results were what we expected them to be, the *mecA* gene was positive for samples 9-13 and negative for samples 14 and 15 (**Figure 3.4**). However, there were no types observed. We believed this was possibly due to a lower quality gel imager and decided to repeat the run. Our findings regarding the *mecA* gene were consistent, but we observed type IVa in sample 11 and type II in sample 12 (**Figure 3.5**) on our second run. To confirm these findings an identical run was completed a third time. Our findings were consistent with what was found in our second run, but additionally sample 10 was positive for IVd (**Figure 3.6**).

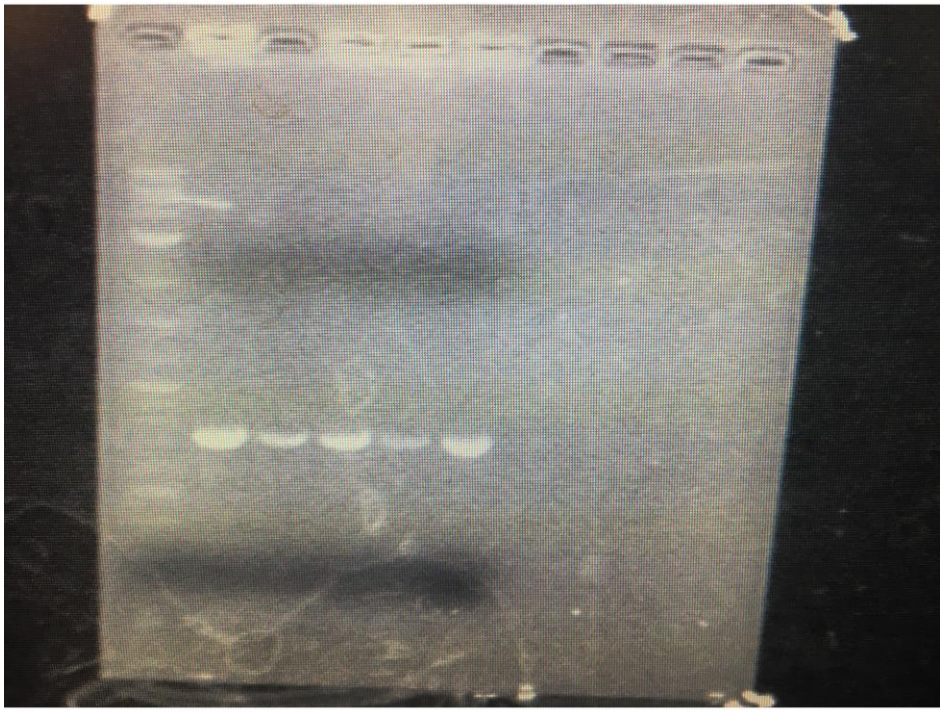


Figure 3.4. Gel of patient samples 9-15 from February 10th, 2017. Primers used are as follows: type I, II, III, IVa, IVb, IVc, IVd, V and *mecA*1417. The *mecA* gene is positive for samples 9, 10, 11, 12, and 13. The *mecA* gene is negative for samples 14 and 15.

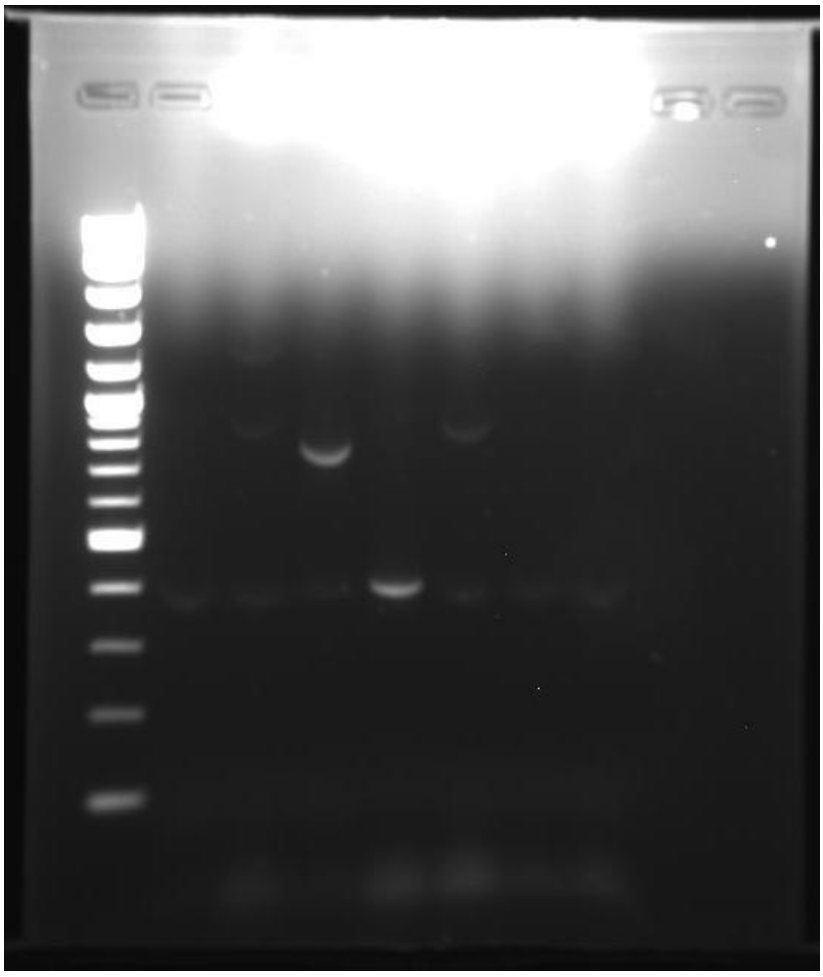


Figure 3.5. Gel of patient samples 9-15 from February 20th, 2017. Primers used are as follows: type I, II, III, IVa, IVb, IVc, IVd, V and *mecA*1417. The *mecA* gene is positive for samples 9, 10, 11, 12, and 13. The *mecA* gene is negative for samples 14 and 15. In patient sample 11 a strong band is seen at ~800 bp, indicating type IVa. In patient sample 12 a strong band is present at ~400 bp, indicating type II.

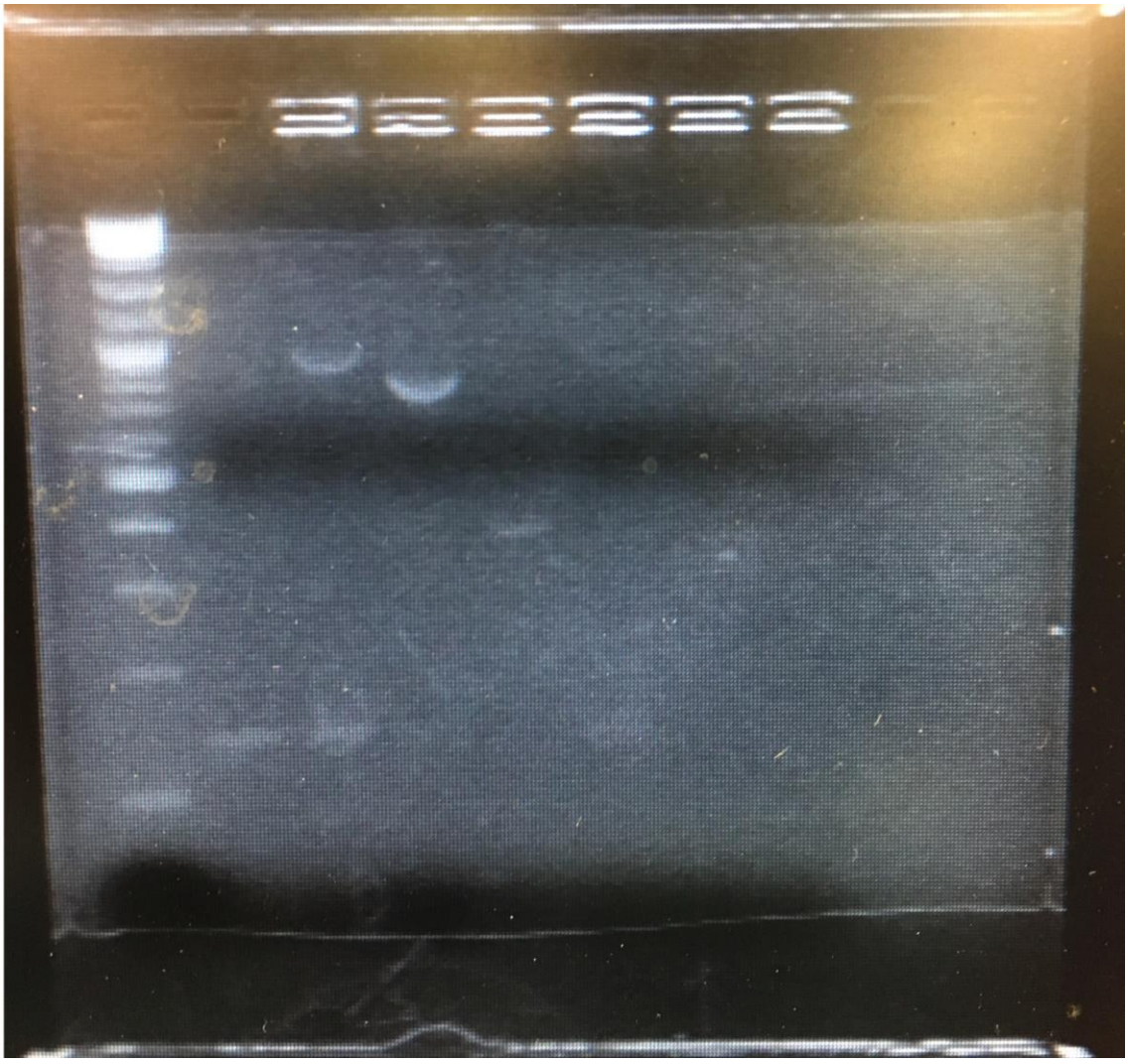


Figure 3.6. Gel of patient samples 9-15 from February 25th, 2017. Primers used are as follows: type I, II, III, IVa, IVb, IVc, IVd, V and *mecA*1417. The *mecA* gene is positive for samples 9, 10, 11, 12, and 13. The *mecA* gene is negative for samples 14 and 15. In patient sample 10 a strong band is seen at ~900 bp, indicating type IVd. In patient sample 11 a strong band is seen at ~800 bp, indicating type IVa. In patient sample 12 a strong band is present at ~400 bp, indicating type II.

Although patient samples 14 and 15 were labeled as MSSA, not MRSA, by Erlanger hospital we wanted to confirm that they were not MRSA. Testing for the *mecA* and mec_{LGA251} would allow us to confirm this. Samples 14 and 15 tested negative for both *mecA* and mec_{LGA251}, from this we determined they were in fact MSSA (**Figure 3.12**). We also streaked patient samples 14 and 15 on CHROM MRSA agar, since it is a good indicator of MRSA with the *mecA* gene (**Section 3.5**).

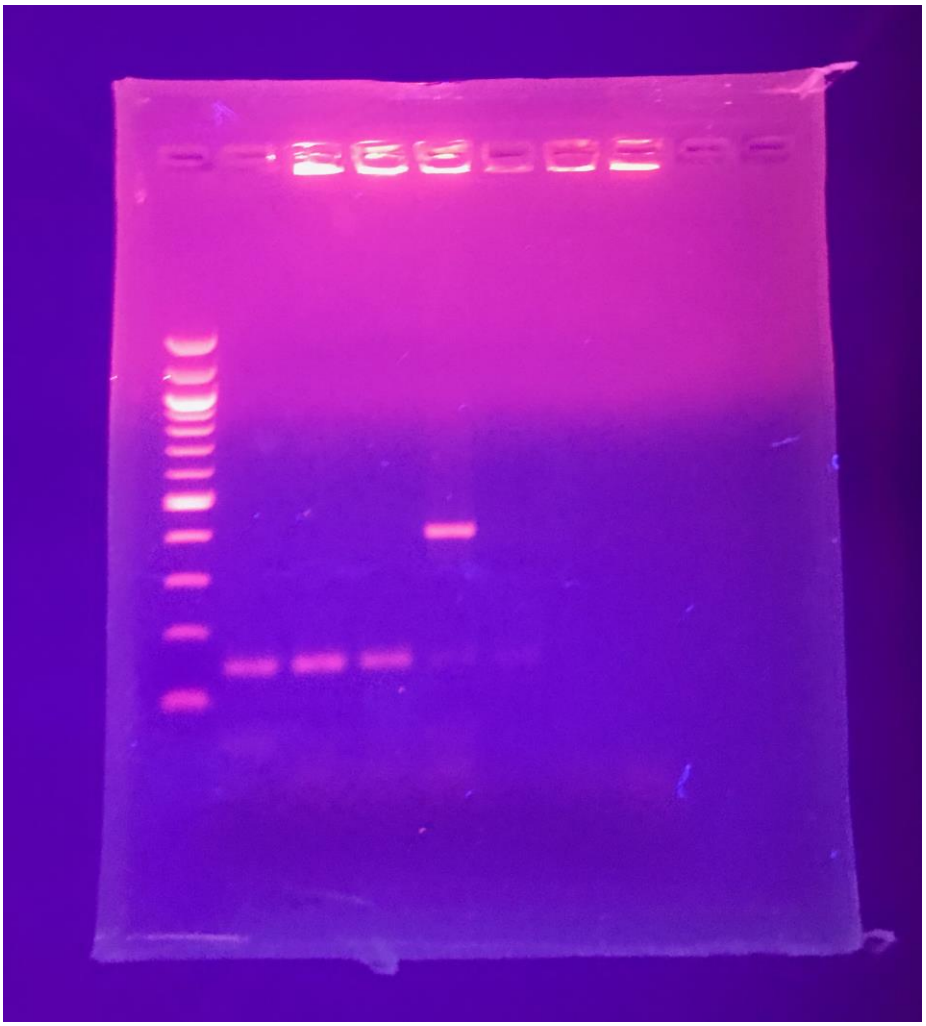


Figure 3.7. Gel of patient samples 9-15 from November 8th, 2017. Primers used are as follows: type I, II, III, IVa, IVb, IVc, IVd, V mecA1417, and mec_{LGA251}. The *mecA* gene is positive for samples 9, 10, 11, 12, and 13. The *mecA* and mec_{LGA251} gene is negative for patient samples 14 and 15. In patient sample 12 there is a strong band at ~400 bp, indicating type II.

We completed a large gel consisting of patient samples 1-15. Samples 4, 8, 14, and 15 were negative for *mecA*, which was expected. We tested positive for types II and IVd, but did not test positive for IVa. This could have been due to various reasons (**Figure 3.8**).

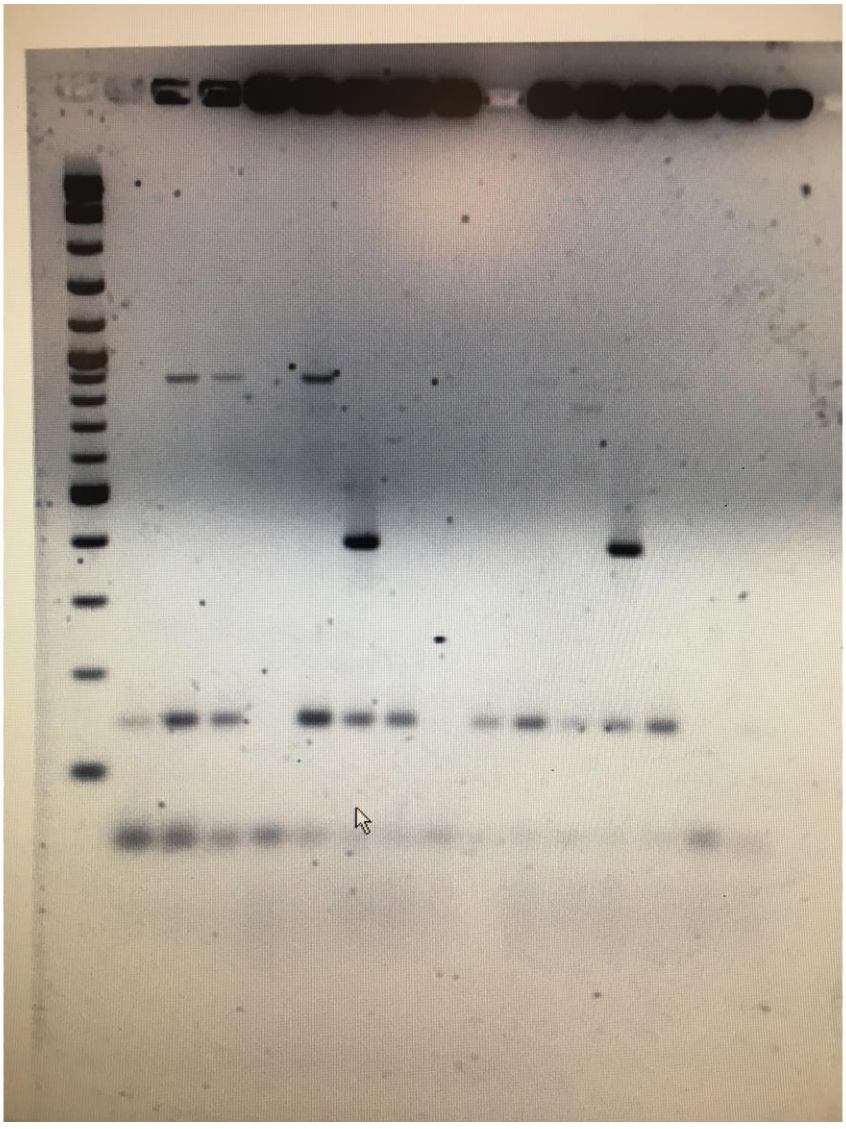


Figure 3.8. Gel of patient samples 1-15 from February 22nd, 2017. Primers used are as follows: type I, II, III, IVa, IVb, IVc, IVd, V and *mecA*1417. The *mecA* gene is positive for samples all samples except 4, 8, 14, and 15. In patient samples 2, 3, and 5 a strong band is present at ~880 bp, indicating type IVd. In patient samples 6 and 12 a strong band is present at ~400 bp, indicating type II.

In December 2017 we received ~50 new patient samples. After preforming genomic extractions on our new samples we were able to run multiplex PCR and gels on them. We tested patient samples 18-25 with our standard set of primers (*mecA*1417, types I-IVd), but left out type V (~325 bp) so we could observe if TSST (Toxic Shock Syndrome Toxin, a virulence factor) was present (~326 bp). The two are too close in size to differentiate if a sample was to test positive. All samples were positive for *mecA*. No samples were positive for TSST (**Figure 3.9**).

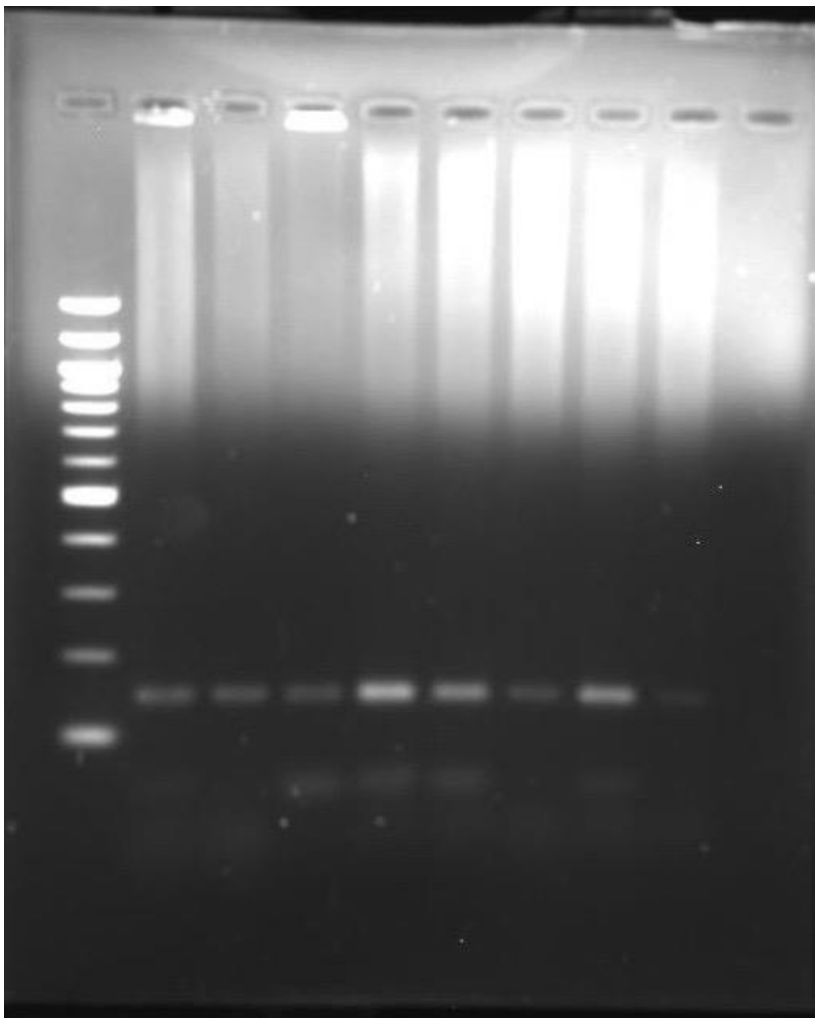


Figure 3.9. Gel of patient samples 18-25 from February 16th, 2018. Primers used are as follows: type I, II, III, IVa, IVb, IVc, IVd, *mecA*1417, and TSST. The *mecA* gene is positive for samples. No samples were positive for TSST.

We repeated this run, but used type V instead of the TSST primer. Our results regarding *mecA* were consistent, but we also had patient samples 22 and 23 test positive for type III at ~280 bp. No samples were positive for type V (**Figure 3.10**).

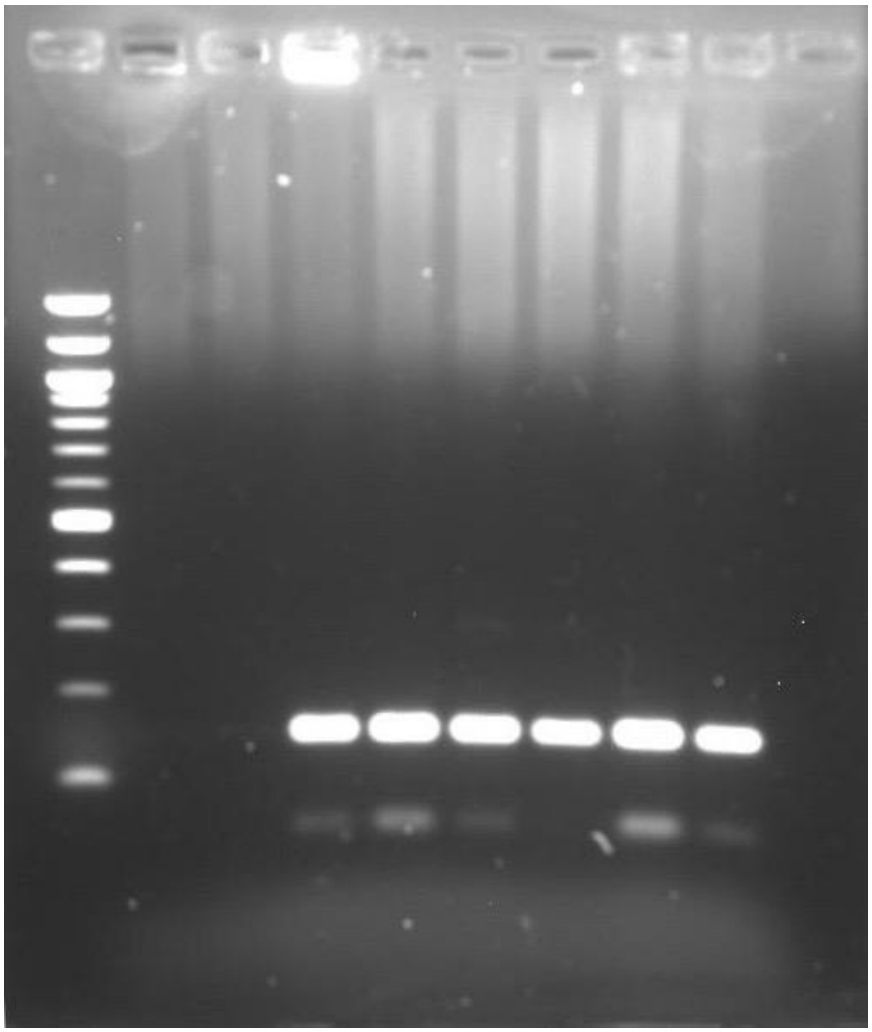


Figure 3.10. Gel of patient samples 18-25 from February 23rd, 2017. Primers used are as follows: type I, II, III, IVa, IVb, IVc, IVd, V and *mecA*1417. The *mecA* gene is positive for all samples. samples 22 and 23 a band is present at ~280, indicating type III.

3.3 Multiplex PCR Characterization of Environmental Samples

Environmental samples, 1c-8c, were observed using 2 sets of primers. In lanes 1-8 we used our standard set of primers (*mecA*1417, types I-V) and in lanes 9-16 we used unique mix found in a different paper. In lanes 1-8 there *mecA* gene was positive for samples 4-8, but we decided to run another gel in hopes to see stronger bands in samples 1-3. Additionally, sample 4 was positive for type IVa (**Figure 3.11**).

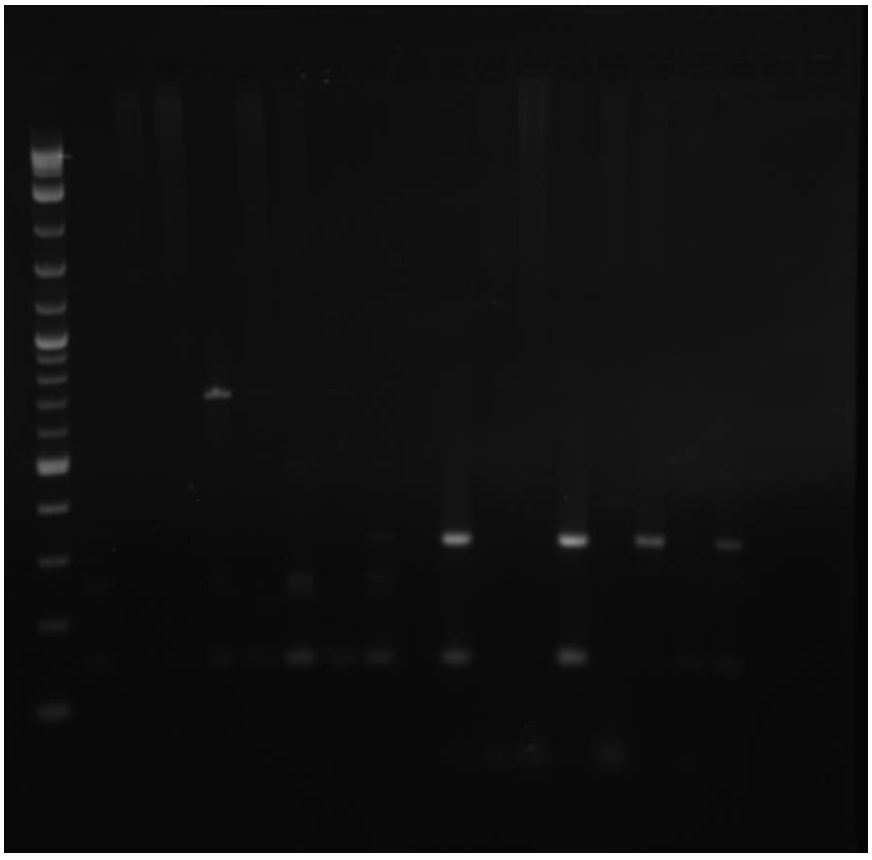


Figure 3.11. Gel of environmental samples 1c-8c from March 29th, 2017. Two sets of primers were used. In lanes 1-8 the primers used are as follows: type I, II, III, IVa, IVb, IVc, IVd, V and *mecA*1417. In lanes 9-16 the primers used are as follows: *mecA*1417, *mecI,* ISI272, ccrAB-α2, ccrAB-α3, ccrAB-α4, ccrAB-β2, and ccrC. In environmental sample 4 a strong band is seen at ~800 bp, indicating type IVa. In lanes 9, 12, 15, and 16 bands are present at ~146 bp, indicating *mecA*. In lanes 9, 12,14, and 16 bands are present at~336 bp, indicating ccrC gene which is harbored by MRSA. [26,34]

A second gel was run on environmental samples 1c-8c again, this time only using 1 set of primers (*mecA*1417, types I-V). Samples 1c and 4c-8c were positive for *mecA*. Samples 2c and 3c were negative for *mecA* despite being previously determined as MRSA by Dr. Spratt's lab. Sample 4c was positive for type IVa. Sample 6c was positive for type III. Sample 8c is positive type V (**Figure 3.12**).

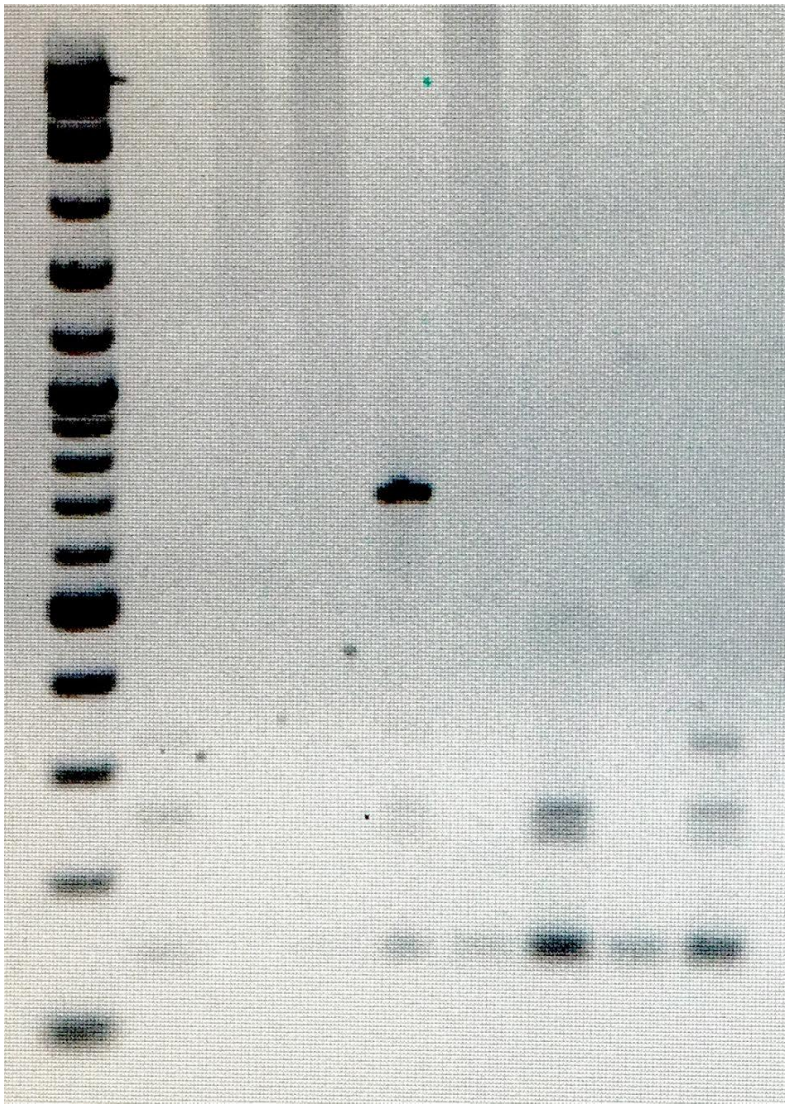


Figure 3.12. Gel of environmental samples 1c-8c from September 22nd, 2017. Primers used are as follows: type I, II, III, IVa, IVb, IVc, IVd, V and *mecA*1417. In environmental sample 4c a strong band is present at ~800 bp, indicating type IVa. In environmental sample 6c a strong band is present at ~280 bp, indicating type III. In environmental sample 8c a strong band is present at ~325 indicating type V.

Since environmental samples 2c and 3c were expected to be MRSA, we did a third run suspecting the two samples would be positive for *mecA*. Additionally, we used PVL (Panton-Valentine Leukocidin, a virulence factor,~433 bp) and TSST (~326 bp) primers in place of types IVb (~493) and V (~325), respectively. The *mecA* gene was present in all samples. No samples were positive for PVL or TSST (**Figure 3.13**).

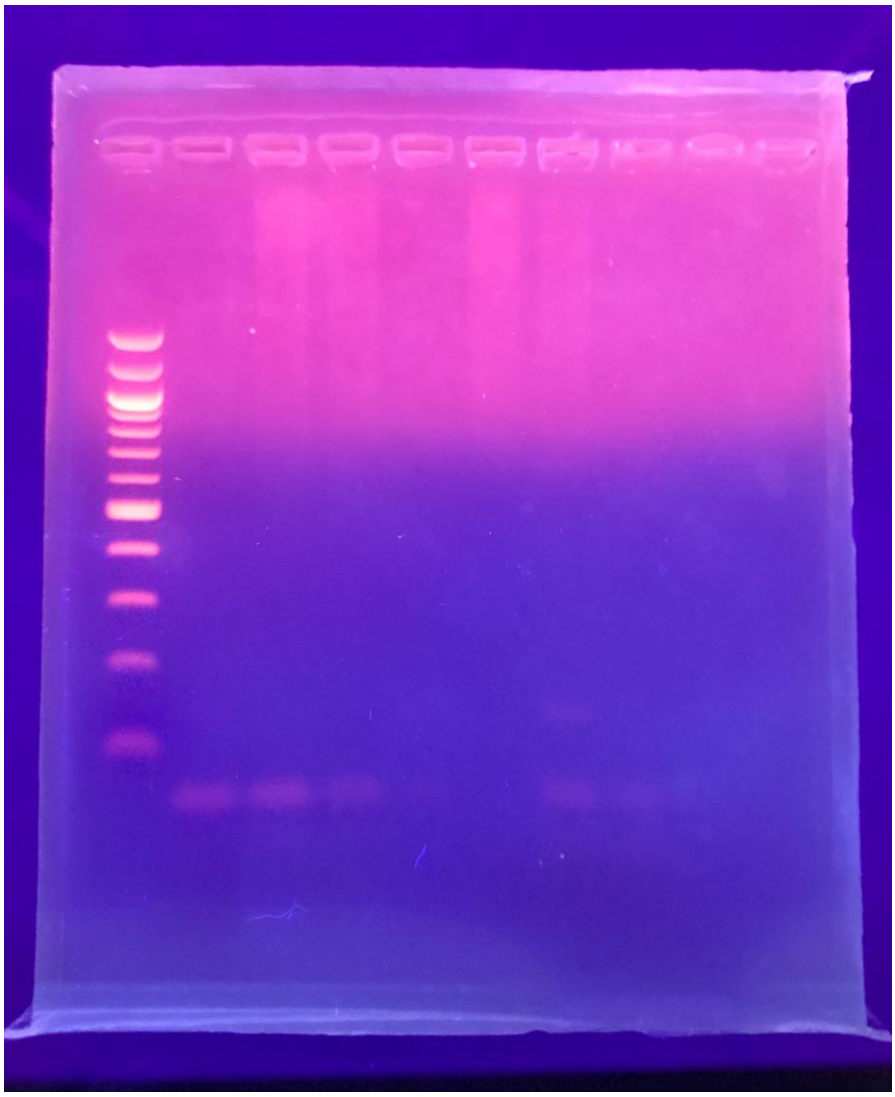


Figure 3.13. Gel of environmental samples 1c-8c from December 2nd, 2017. Primers used are as follows: type I, II, III, IVa, IVc, IVd, *mecA*1417, PVL, and TSST. Type IVb and V were not tested for as their bp are too close in size to PVL and TSST to differentiate. No environmental samples tested positive for PVL or TSST.

3.4 Multiplex PCR Characterization of Control Samples

Later into the study, we purchased control samples of MRSA from the American Type Culture Collection to ensure our primers were operating properly. We purchased BAA-41, BAA-2094, and 33592, which were controls for types II, III, and V, respectively. We ran a PCR and gel with primers for type II, III, V, and *mecA*1417. BAA-41 and BAA-2094 did not test positive for their expected types. This could be due to human error and will be repeated at a later date (**Figure 3.14**).

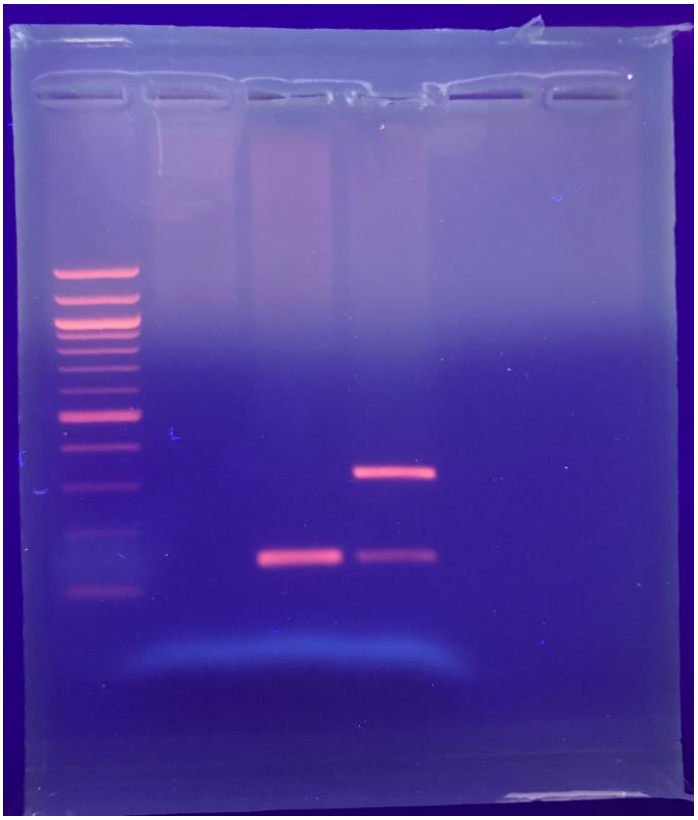
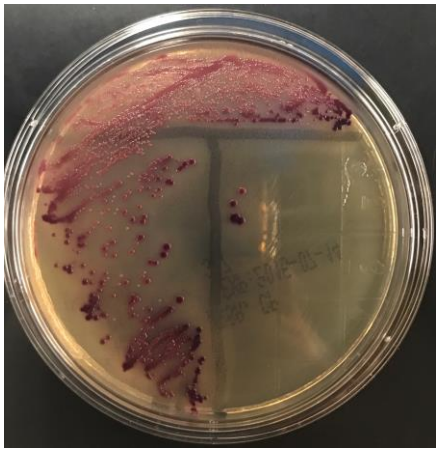


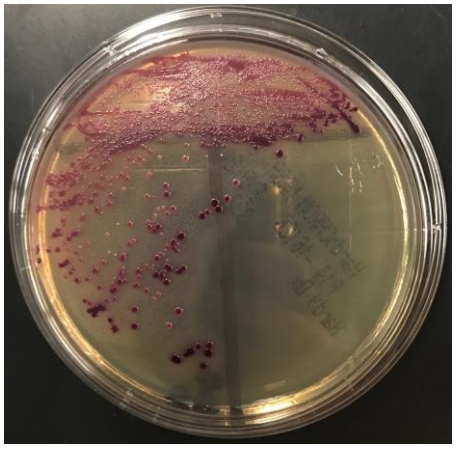
Figure 3.14. Gel of control samples (BAA-41, BAA-2094, and 33592) from March 6th, 2018. Primers used are as follows: type II, III, V, and *mecA*1417. The *mecA* gene is positive for samples BAA-2094 and 33592. Sample 33592 was positive for type V, which was expected. BAA-41 and BAA-2094 were negative for, respectively, type II and III, which was not expected.

3.5 Plates

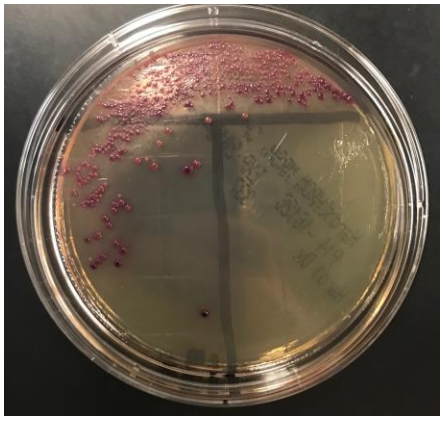
After repeated multiplex PCR and gels to identify *mecA* or *mec*$_{\text{LGA251}}$ in patient samples 4 and 8 were consistently negative we decided to streak plates and compare them to known MSSA samples, samples 14 and 15. Working with Colin Smith, utilizing visual examination of the plates combined with repeated negative results from gels we determined samples 4 and 8 were also MSSA, not MRSA.



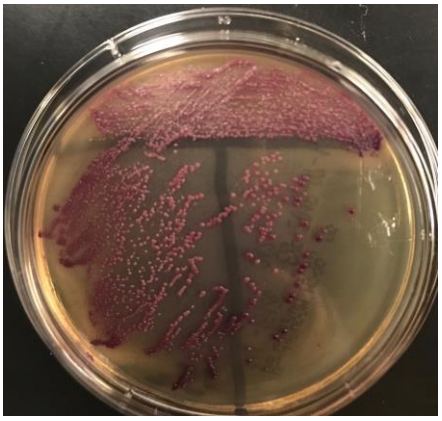
Patient Sample 4



Patient Sample 8



Patient Sample 14



Patient Sample 15

Figure 3.15. Patient samples 4, 8, 14, and 15 streaked onto agar plates were determined to be methicillin-sensitive *S. aureus.*

3.6 Pie Graphs

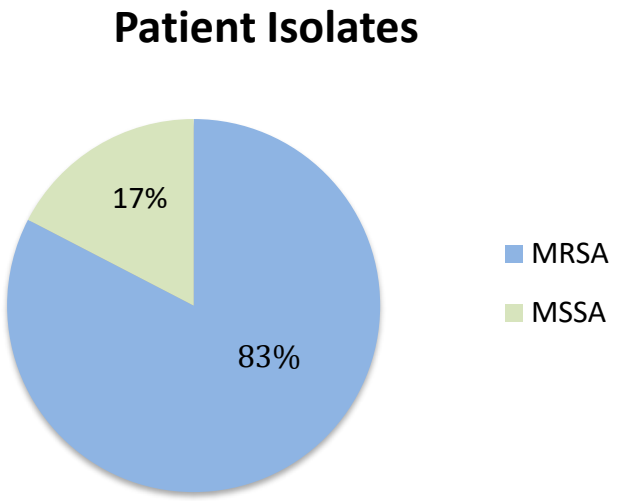


Figure 3.16. Comparison of MRSA and MSSA patient isolates.

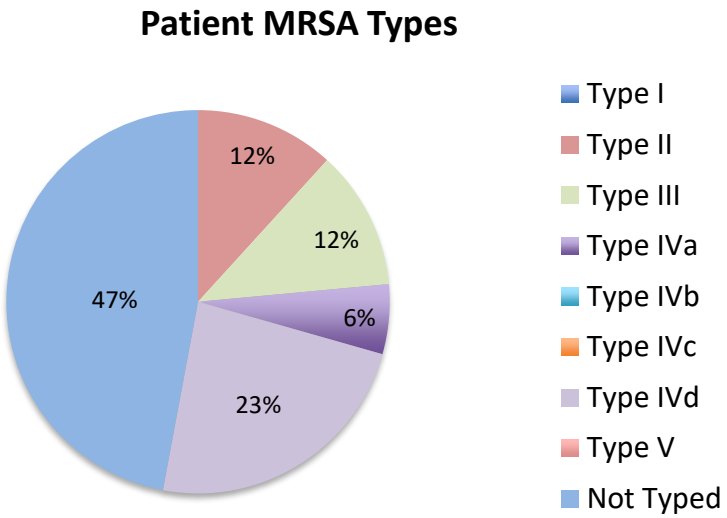


Figure 3.17. Types found within MRSA patient isolates.

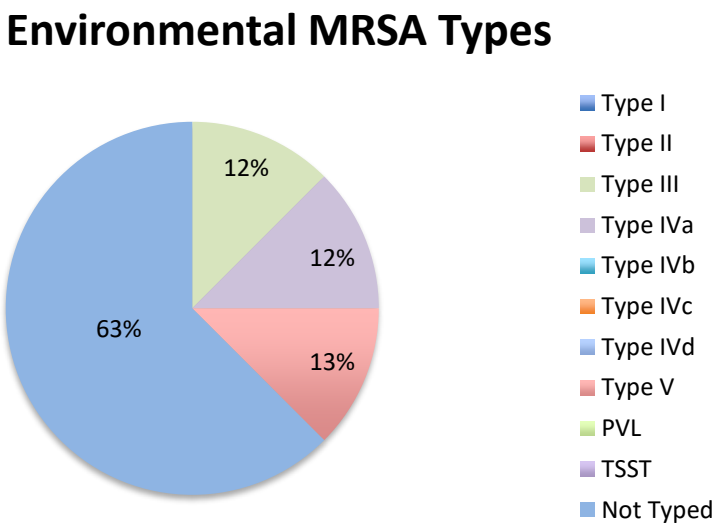


Figure 3.18. Types found within MRSA patient isolates.

Chapter 4

Discussion and Future Direction

Methicillin resistant *S. aureus* is a major public health concern. A patient infected with MRSA can experience many complications, with some even being life threatening. Due to this bacteria's severity, it is imperative for researchers to have a thorough understanding of how it is transferred, different types present, if it is carrying virulence factors, and so forth.[58]

We had multiple goals in this research: to confirm if isolates were MRSA or not, identify if there was any correlation between patient and environmental isolates, and to determine if any isolates had virulence factors.

Beginning with our patient samples we used a standard set of primers consisting of the following: type I, II, III, IVa, IVb, IVc, IVd, V and *mecA*1417. The *mecA*1417 gene is indicative of MRSA allowing us to confirm if isolates were MRSA or not. Patient samples 4, 8, 14, and 15 were all negative for *mecA*1417. We were not surprised samples 14 and 15 were negative as they were labeled methicillin-sensitive, not resistant, *S. aureus* (MSSA) when we received them. However, patient samples 4 and 8 were labeled MRSA, which prompted us to rerun our PCR and gel. Again we had negative results for *mecA*1417 with patient samples 4 and 8, but it was possible that these samples had the divergent gene for *mecA*, known as $mecA_{LGA251.}$ Patient samples 4 and 8 also tested negative for $mecA_{LGA251}$ leading us to believe they were not MRSA, but instead MSSA. We streaked plates with our two

known methicillin-sensitive samples, 14 and 15, and what we believed to be methicillin-sensitive samples, 4 and 8. After allowing these samples to grow and upon visual examination it was determined samples 4, 8, 14, and 15 were MSSA.

Our patient samples exhibited various types of MRSA. Patient samples 2, 3, 5, and 10 were positive for type IVd. Patient samples 6 and 12 were positive for type II. Patient sample 11 was positive for type IVa. Patient samples 22 and 23 were positive for type III. Patient samples 16 and 17 were contaminated during our process and no longer viable for observation. The remaining MRSA patient samples: 1, 9, 18, 19, 20, 21, 24, and 25 were not typed. Due to time constraints, we only observed types I-V, but there is typing available up to type X.[59,60]

Our 8 environmental samples were all positive for *mecA*1417. Environmental sample 4 was positive for type IVa. Environmental sample 6 was positive for type III. Environmental sample 8 was positive for type V. The remaining MRSA environmental samples: 1, 2, 3, 5, and 7 were not typed, but may be types VI-X that were not tested for. Types III and IVa were present in both patient and environmental samples. This could indicate transmission from various objects or surfaces to patients.[60]

Our most common type of MRSA found was type IV. Type IV MRSA has also been found in other countries such as Brazil, Denmark.[61,62] Additionally in other studies where U.S. isolates were observed, type IV isolates were a common finding.[63]

The limiting factor to this study was the small amount of environmental samples available. If there were a larger amount of environmental samples it is

possible that additional correlations would have been found between patient and environmental samples.

In future studies researchers should examine the 42 new patient samples we have received from Erlanger hospital's NICU and PICU. These should be examined for *mecA*1417, *mecA*$_{LGA251}$, and type I-X. Additionally, more environmental samples should be obtained with the known location (stethoscope, crib, air duct, floors, etc.) in order to observe more detailed correlations between patient and environmental samples, as well as identify if certain locations have higher incidences of a certain type or virulence factors than others.

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