Investigation of *Staphylococcus aureus* Bacteriophage Population at a South Carolina University: The Disappearance of *S. aureus* Bacteriophage Population Amidst the COVID-19 Pandemic

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Bacteriophages are naturally occurring, nonpathogenic viruses, which infect bacterial cells. Recently, bacteriophage research has increased with hopes of using them against antibiotic resistant bacterial infections in the future. This study aimed to determine a possible correlation between perceived stress and the *Staphylococcus aureus* bacteriophage population at Coastal Carolina University (CCU), Conway, South Carolina, using isolation and characterization techniques to further understand humans as a potential bacteriophage source. From October 2020 to March 2021, nasal and postauricular swab samples were collected from 12 participants on a monthly basis along with a perceived stress survey. Samples were subjected to filtration, amplification, plaque assays, and PCR techniques to identify and characterize bacteriophage. The purpose of this study was to understand humans as a repository for bacteriophage and to understand factors, namely perceived stress, which affect bacteriophage presence on humans. Results suggested that possible changes due to the COVID-19 pandemic, such as increased stress levels, mask wearing, and constant hand washing/ sanitizing, caused a drastic decrease in the *Escherichia coli* and *Staphylococcus aureus* phage population at Coastal Carolina University.

Introduction

Bacteriophages, also known as phages, are naturally occurring viruses, which are non-pathogenic to humans, and occupy bacterial cells as their hosts [1]. Phage make up approximately 10^{31} particles on earth, thus their role in maintaining microbial homeostasis in all environments is crucial [2]. Bacteriophages do so by utilizing the lytic life cycle, thus targeting specific strains of bacterium as their reproductive host, and eventually causing cell lysis. After bacterial cell lysis, bacteriophages are released into the environment to infect more bacterial cells [3]. Their high specificity and potential for self-dosing have led to their widespread study after their existence was first discovered by Frederick Twort in 1915 and Felix d'Herelle in 1917. d'Herelle, shortly after discovery, went on to use phage therapy to treat dysentery in 1919, in Paris, France [1]. In 1921, Richard Bruynoghe and Joseph Maisin used phage therapy to treat and cure staphylococcal skin infections [1]. Despite these early successes, by the 1940's most phage therapy research was marginalized by the discovery of antibiotics, namely penicillin. Bacterial antibiotic resistance was observed shortly thereafter and has caused a crisis in the medical community since [4].

This study aims to better understand the nature of *S. aureus* phage and its possible correlation to perceived stress levels. Better understanding the nature of *S. aureus* phage would aid in understanding human repositories which could be used as phage treatment for the antibiotic resistant *S. aureus* variant methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA has rapidly posed a threat to the general wellbeing of the public. From 1999-2005, the number of hospitalizations due to MRSA have increased by more than 50%, thus causing MRSA to be at endemic, and even epidemic levels at many hospitals in the United States [5]. Since then, healthcare officials have given the upmost importance to decreasing hospitalizations due to MRSA, especially since most patients contract MRSA from hospitals or other healthcare settings [6]. The numbers of hospital acquired MRSA bloodstream infections have been declining for the past eleven years and have seen a 74% decrease in cases. Community associated MRSA (CA-MRSA) infections have also been declining at a rate of approximately 3.9% per year for the past eleven years [7]. Despite the recent reductions in cases, the decline in CA-MRSA infections is due to increased diligence in health care related sectors of the community [6]. This can be seen as CA-MRSA cases, not including healthcare settings within the community, has only decreased from 5.9 to 5.2 infections per population of 100,000 from 2005 to 2011. Outside of healthcare settings, CA-MRSA now accounts for 14% of MRSA cases, as of 2011. This number is projected to increase [6]. Since the decrease in CA-MRSA is typically only seen in healthcare settings, this still leaves high school, college, and professional athletes, men who have sex with men, military personnel, prison inmates, and children in day care centers at risk of developing CA-MRSA [8]. CA-MRSA has been causing skin/soft tissue infections, osteomyelitis, sepsis, endocarditis, toxic shock syndrome, and necrotizing pneumonia in seemingly healthy individuals [9]. This poses a dilemma as treatments for CA-MRSA are elusive as methicillin, linezolid, vancomycin, teicoplanin, and daptomycin are increasingly ineffective against MRSA [10]. MRSA's antibiotic resistance stems from bacteria's ability to mutate rapidly at an approximate rate being 1 in 10^5 to 10^8 [11]. Therefore, it is pertinent that new treatments are developed and/or refined to be used in the fight against antibiotic resistant bacterial infections such as MRSA.

Bacteriophages have the potential to be naturally sourced from the human population for potential phage therapy. In order to do so, the bacteriophage population on humans must be further understood. From 2014-2018, 225 samples were taken from students and faculty at Coastal Carolina University to determine *S. aureus* and *E. coli* B phage presence. Of the 225 aforementioned samples, it was determined that 42.9% were lytic to *S. aureus* and 36.8% were lytic to *E. coli*. In 2018 another pilot study was done in Dr. Paul E. Richardson's lab to track phage presence throughout the year. In this study, samples from 15 participants were collected on a monthly basis starting in October 2017 and ending in March 2018. Results determined that throughout the year the coliphage and *S. aureus* phage presence fluctuated, with coliphage and *S. aureus* phage presence having a seemingly inverse relationship due to temporal competition [12].

In this study 12 participants provided samples from behind their ear and in the entryway of the nasal passage on a monthly basis. Sterile filtration and amplification techniques were used to grow potential bacteriophage in samples. Microbial tests were performed to determine the presence of bacteriophages which were lytic to *S. aureus* and *E. coli*. Molecular techniques were used to determine the identity of specific phage groups. Furthermore, a subjective stress survey was created for the October 2020-March 2021 collections. This survey was taken by participants monthly to determine a potential correlation between perceived stress level and bacteriophage presence. Immunosuppression is often linked to high stress levels [13]; therefore, it can be hypothesized that as stress levels increase, *S. aureus* becomes more prevalent and consequently so does *S. aureus* phage. The purpose of this study was to understand humans as a repository for bacteriophage and to understand factors, namely perceived stress, that affect bacteriophage presence on humans. This led to the following two questions: Does the presence of bacteriophage on 12 students and faculty at Coastal Carolina University change throughout the year? Can a correlation between the perceived stress level of the participant and bacteriophage presence be determined?

Methods

Postauricular and nasal samples were collected from 12 participants on a monthly basis. Samples were collected by swabbing a sterile cotton swab near the opening of the participant's nasal passageway. The end of the swab was then broken off into a 2 mL microcentrifuge tube (Carolina Biological Supply Company) containing 1000 µL of phosphate buffered saline (PBS) (PBS Tablets, Calbiochem). The same technique was performed on the participant again, but this time the participant was swabbed postauricularly, directly behind the ear. To follow the COVID-19 protocols set up by Coastal Carolina University all samples were collected by the participants, with the research assistants only providing verbal cues to assist the volunteers. Along with the sample collection, participants took a subjective stress survey, as shown in Figure 1. The sample incubated in the PBS solution for thirty minutes at room temperature. The samples were then placed in a syringe with a 0.45-micron PTFE membrane filter (VWR International) attached to a 10 mL Luer-Lok Tip syringe (BD) and filtered into a new microcentrifuge tube.

Amplification of Viral Particles

Amplification of the samples was performed using *E. coli* B, *E. coli* K12, and *S. aureus* cultures (Carolina Biological Supply Company). In a 15 mL culture tube (Thermo Fisher) approximately 3.0 mL of LB Miller Broth (Sigma-Aldrich) and 200 µL *E. coli* B culture was combined and incubated at 37 ℃ for 30 minutes. One hundred microliters (100 µL) of the filtered sample were added to the culture tube after the incubation period. The sample was then incubated overnight on a shaking incubator at 37℃. This was repeated for each sample with *E. coli* K12 and *S. aureus*.

Plaque Assays

One hundred microliters (100 µL) of *E. coli* B culture were added to an LB Agar plate and sterile spreading techniques were used to create a bacterial lawn. This was incubated for 20 minutes at 37℃. Then, 5 µL of sample were added to three designated areas of the plate. In the fourth designated area *E. coli* B served as a negative control. The plates were then incubated overnight at 37℃. The following day possible plaque formation was observed. This was repeated using *E. coli* K12 and *S. aureus* as the bacterial hosts.

DNA Extraction

Samples which had plaque formation or non-determinant plaque formation were subjected to DNA extraction. One hundred microliters (100 µL) of amplified sample were placed into a 2.0 mL microcentrifuge tube and centrifuged at 2,500 rpm for 5 minutes. The supernatant was transferred to a new microcentrifuge tube along with 5 µL of proteinase K (Thermo Fisher). The sample was incubated at room temperature for 60 minutes. After room temperature incubation, the sample was incubated at 95℃ for five minutes.

Polymerase Chain Reaction

Polymerase chain reaction was conducted by adding 25 µL of Gotaq Green Master Mix (Promega Corporation), 21 µL template (DNA extraction), and 4 µL of the corresponding primer set in a 0.2 mL PCR tube (VWR International). PCR analysis was conducted in the Bio-Rad T100 thermal cycler. PCR analysis began with a 4 minute DNA unwinding step at 95°C, followed by 39 cycles of DNA denaturation (30 seconds at 94°C), primer annealing (1 minute at 55°C for) , and DNA extension (72°C for 2 minutes). Final PCR products were held at 4°C for short-term storage. Positive and non-determinant *S. aureus*, *E. coli* B, and *E. coli* K12 phage samples were subjected to PCR analysis. Primer set for Coliphage comprised of CPA, CPB, and CPO; primer sets for *S. aureus* phage comprised of SPA and SPB as indicated in Table 1.

Gel Electrophoresis

Gel electrophoresis was performed on samples subjected to PCR analysis. 2% agarose (Agarose I, VWR) gels using 1x Tris-acetate EDTA (TAE) buffer were used to image the PCR products. 5µL of ethidium bromide was used as a staining agent for the gel. Ten microliters (10 µL) of 1 kb DNA ladder (Promega Corporation) and 10 µL of PCR product were loaded into their respective wells. The gels were run at 60 volts for 120 minutes before being imaged under UV light with the Molecular Imager ChemiDoc XRS+ Imaging System from BioRad Laboratories, Inc.

Stress Survey

The Bacteriophage Therapy Project

This survey is for research purposes only. Please answer each of the questions to the best of your ability. If you do not feel comfortable answering any of the questions below, please notify the person administering the survey. The first four questions you must answer on a scale of 1-10, with one being you feel almost no stress at all and 10 being you are overwhelmed with stress. The last two questions answer to the best of your ability.

1. How much stress do you feel overall this week?

1 2 3 4 5 6 7 8 9 10

2. How much stress do you feel due to online classes this week?

1 2 3 4 5 6 7 8 9 10

3. How much stress do you feel due to F2F classes this week?

1 2 3 4 5 6 7 8 9 10

4. How much stress do you feel due to your social life this week?

1 2 3 4 5 6 7 8 9 10

5. How much stress do you feel due to changes in everyday life of COVID on campus, i.e., wearing a mask, 6 ft apart, changes in exits/entrances, etc.?

1 2 3 4 5 6 7 8 9 10

6. Do you feel your stress level has increased due to COVID changes? (1 not at all and 10 increased a lot)

1 2 3 4 5 6 7 8 9 10

7. Please let us know if you have had or will have a quiz or test:

	Last Week	This Week	Next Week
Test	Yes or No	Yes or No	Yes or No
Quiz	Yes or No	Yes or No	Yes or No

8. Have you felt any physical symptoms of stress in the last week:

Stomach aches	Yes or No
Headaches	Yes or No
Low energy	Yes or No
Chest pains	Yes or No
Muscle Tension	Yes or No

Figure 1: Subjective stress survey developed by Dr. Paul E. Richardson's lab and used to determine a correlation between perceived stress level and bacteriophage presence.

Primers

Coliphage primers consisted of CPA, CPB, and CPO. CPA and CPB primers comprised of individual primers K1F, 933, Micro, T4, HK, Mu, N4, JK, and Lambda were obtained from a 2009 UNC Chapel Hill dissertation by Hee Suk Lee [14]. The CPO primers sets were cultivated by Dr. Paul E. Richardson's lab at Coastal Carolina University. CPO comprised of ORF23 and ORF43. *S. aureus* primers included SPA and SPB which comprised of 3A-Like, TwortLike, 11-Like, and 77-Like, which were derived from R. Pantůček, et al. [15]. Coliphage primer set CPA comprised of 0.2 µL HK Forward ("For"), 0.2 µL HK Reverse ("Rev"), 0.2 µL 933For, 0.2 µL 933Rev, 0.2 µL T4For, 0.2 µL T4Rev, 0.2 µL MicroFor, 0.2 µL MicroRev, 0.2 µL K1FFor, 0.2 µL K1FRev, along with 2.0 µL nuclease-free water (Promega Corporation). Coliphage primer set CPB comprised of 0.22 µL MuFor, 0.22 µL MuRev, 0.22 µL Lambda For, 0.22 µL Lambda Rev, 0.22 µL JKFor, 0.22 µL JKRev, 0.22 µL N4For, 0.22 µL N4Rev, along with 2.22 µL of nuclease-free water. Coliphage primer set CPO comprised of 0.67 µL ORF23 For, 0.67 µL ORF23 Rev, 0.67 µL ORF43 For, 0.67 µL ORF43 Rev, and 1.33 µL of nuclease-free water [16]. *S. aureus* primer set SPA comprised of 0.5 µL 3A-Like For, 0.5 µL 3A-Like Reverse, 0.5 µL Twort Forward, 0.5 µL Twort Reverse, and 2.0 µL of nuclease-free water. The S. aureus primer set SPB comprised of 0.5 µL 11-Like Forward, 0.5 µL 11-Like Reverse, 0.5 µL 77-Like Forward, 0.5 µL 77-Like Reverse, and 2.0 µL of nuclease-free water. The above CPA, CPB, CPO, SPA, and SPB primers each totaled 4.0 µL to be used individually in the PCR reactions.

Table 1: Primer sets CPA and CPB were derived from the dissertation of Hee Suk Lee [14]. Primer set CPO was created by Dr. Paul E. Richardson [16], the principal investigator of this study. Primer set SPA and SPB were derived from R. Pantůček, et al [15].

Primer Set	Target Family/ Organism	Gene Target	PCR Fragment Length (bp)	Primer Name	Primer Size (bp)	Primer Sequences (5' to 3')
CPA	*Podoviridae*	CKV1F, gp34	2110	K1FFor	16	TGGAAGCCCGTGAGAC
				K1FRev	18	GCAGCGTCAATCGCTCGG
	Podoviridae	933Wp09, *hkaG* gene	488	933For	18	GCAATACATCAAACGCCG
				933Rev	16	GCGAATGCCAGCGGCG
	Microviridae	Hypothetical protein	1039	MicroFor	25	GCTGCCGTCATTGCTTATTATGTTC
				MicroRev	25	GYTAYCGBMMCATYAAYTAHTCACG
	Myoviridae	Major head protein (gene 23)	704	T4setFor	20	GATATTTGTGGYGTTCAGCC
				T4setRev	24	GTCAAATACACCAGCTTTAGAACC
	Siphoviridae	cII protein	177	HKsetFor	20	CACAGCGAGAAATTGATCGC
				HKsetRev	19	CTAATCGGACTGATGTCTG
CPB	*Myoviridae*	Tail fiber gene (MUP49)	171	MusetFor	21	GAAAACGACTCAATCCTTGCC
				MusetRev	20	TCATCAGGTCTTTTGTTGTGG
	Podoviridae	Hypothetical protein	2285	N4For	20	GCACATGCAGAATAAGGTTG
				N4Rev	20	CCATTAGTAACACCATCTGC
	Siphoviridae	Tail fiber protein	878	JKsetFor	16	GYGAYCAGATGGTTCC
				JKsetRev	16	CAATRTCYTCYTARTTG
	Siphoviridae	B gene	307	LambdaFor	20	TGGGCGTACTTTATGGGGCG
				LambdaRev	20	CGGACCTGCTGGGCAAAAAT
CPO	Coliphage T2/ T4	ORF 23 (Major capsid protein)	405	ORF23For	20	TGGCGCAGTAACTCAGATTG
				ORF23Rev	20	GCACAGCTTCCATTTGTTT
	Coliphage T2/ T4	ORF 43 (DNA polymerase)	198	ORF43For	20	CCCTGCGCCTTTCATAATAA
				ORF43Rev	20	ATCGCAGGAACAGCTCCTAA
SPA	3A-like phages	Tail Fibers	744	3A-like For	20	TATCAGGCGAGAATTAAGGG
				3A-like Rev	23	CTTTGACATGACATCCGCTTGAC
	Twort-like phages	Major capsid protein	331	Twort-like For	20	TGGGCTTCATTCTACGGTGA
				Twort-like Rev	23	GTAATTTAATGAATCCACGAGAT
SPB	11-like phages	Hypothetical tail proteins	405	11-like For	22	ACTTATCCAGGTGGCGTTATTG
				11-like Rev	23	TGTATTTAATTTCGCCGTTAGTG
	77-like phages	Hypothetical tail proteins	155	77-like For	19	CGATGGACGGCTACACAGA
				77-like Rev	23	TTGTTCAGAAACTTCCCAACCTG

Results

S. aureus Phages

In the 2020-2021 collection year, samples were collected from 12 participants on a monthly basis, therefore a total of 52 samples were collected. Of those 52 samples collected none were lytic to *S. aureus*. Comparably, none of the non-determinant samples analyzed through PCR analysis were positively identified by the SPA or SPB primers

E. coli Phages

During the 2020-2021 collection year, of the 52 samples collected, one sample was lytic to *E. coli* B (1.92%). Of the 44 samples subjected to PCR analysis none of the samples were positively identified by the CPA, CPB, or CPO primers.

Subjective Stress Survey

Participants were instructed to take a stress survey consisting of 8 questions. For questions 1-6 participants were required to rate their perceived stress on a scale of 1-10 with one being the lowest amount of perceived stress and 10 being the most amount of perceived stress. Question one asked participants to rate overall perceived stress, which throughout the year had an average of 6.63. Question two inquired how much perceived stress was felt due to online classes, which had an annual average of 6.06. Question three inquired regarding perceived stress levels due to face-to-face classes, which had an annual average of 4.39. Question four asked regarding perceived stress levels due to social life, which had an annual average of 3.57. Question four inquired regarding perceived stress levels due to COVID-19 changes on campus, which had an annual average of 4.87. Question five inquired regarding perceived increase in stress level due to the COVID-19 pandemic, which had an annual average of 5.73.

Discussion

For five years *S. aureus* and *E. coli* phage monitoring has occurred on the campus of Coastal Carolina University. Throughout the 2014-2018 collections, there was an average of 42.9% and 36.8% phages lytic in microbial testing to *S. aureus* and *E. coli* respectively [12]. From September 2020-March 2021, the most recent collection year, one sample was found to be lytic to *E. coli* B. There were no phages which were lytic to *S. aureus* throughout the year, which is a 100% decrease in *S. aureus* phage and a 94.8% decrease in coliphage from the previous four collection years spanning 2014-2018.

Since the 2014-2018 sample collections, the COVID-19 pandemic emerged. With the emergence of this new virus has come a plethora of changes in human life. Increased stress levels, constant face mask wearing, and consistent hand wash/ sanitizing have become routine. A noted change in the collections between 2014-2018 to the 2020-2021 year has been the disappearance of the bacteriophage population on students and faculty at CCU.

Participants indicated an average perceived increase in stress due to the COVID-19 pandemic of 5.73, on a scale of 1-10. Thus, depicting a high increase in day-to-day perceived stress levels. A correlation was found between anxiety and increased acne severity by a study conducted

by Zari and Alrahmani [17]. Thus, it is possible that as perceived stress levels rose, so did acne on most individuals. Acne is cause by *Propionibacteria* [18]. Pieterse et al., documented changes in the coliphage population and *S. aureus* phage population and found that if one were to increase, then the other decreased [12]. This was most likely due to competition between their respective bacterial strains. Therefore, if another bacterial strain is in a surplus in the microbiome, this could lead to competition and a decrease in *E. coli* and *S. aureus* in the human facial microbiome. Subsequently, this may lead to a decrease in the coliphage and *S. aureus* phage population.

Constant mask wearing has the potential to have a similar effect on the human facial microbiome as anxiety induced acne. Mask wearing not only can cause acne but can also change the microbiome in many ways. During this study samples were collected from behind the participant's ear and in the opening of the nasal passageway, both of which are places the mask covers when worn. A possible change in microbiome was seen in this study as most microbial tests came back as nondeterminate, due to bacteria in the samples passing through the filtration step, as depicted in Figure 2. This bacterial contamination was not seen in previous years, thus this new bacterial strain causing contamination could be instigating temporal competition amongst itself and *S. aureus/ E. coli*. Lower levels of *S. aureus* and *E. coli* would subsequently lead to a decrease in the presence of coliphage and *S. aureus* phage.

Increased hygiene may also play a role in the disappearance of coliphage and *S. aureus* phage. In Dr. Paul E. Richardson's lab, coliphage monitoring on humans occurred as a way to indirectly track fecal contamination on students and faculty at Coastal Carolina University. It was found that 36.8% of participants tested were positive for coliphage, indirectly showing that 36.8% of participants did not wash their hands consistently [12]. Another project in Dr. Paul E. Richardson's lab surveyed students on the campus of CCU to determine COVID-19 habits among college students, faculty and staff. It was found that 95.4% of participants washed their hands frequently, while 88.6% of participants use hand sanitizer frequently (unpublished data from the COVID-19 survey-Dr. Fredanna McGough and Dr. Paul E. Richardson). This is a drastic change from past data regarding fecal contamination. Therefore, an increase in hygiene could impact the presence of bacteriophage on participants.

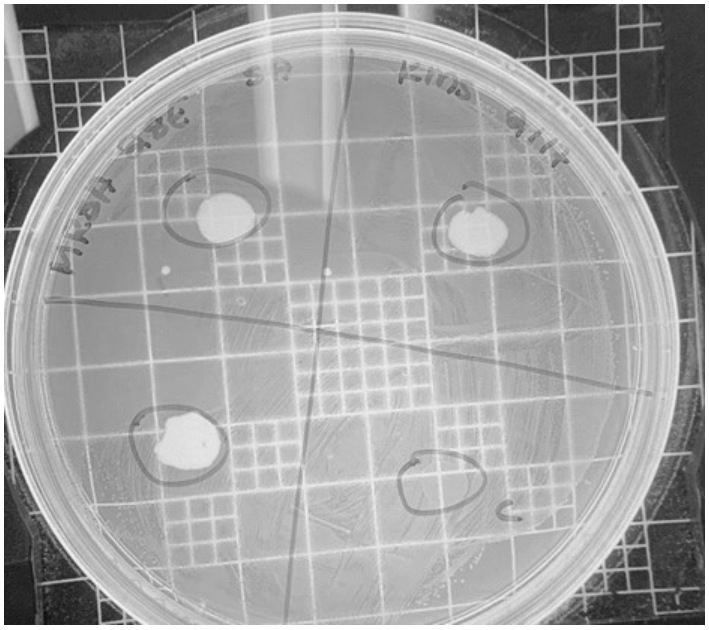

Figure 2: Plaque assay containing bacterial contamination from amplified sample. The circle to the far right contains bacterial culture as a negative control.

Conclusion

The purpose of this study was to understand humans as a repository for bacteriophage and to understand factors, namely perceived stress, which affect bacteriophage presence on humans. The first question in this study was does the presence of bacteriophage on 12 students and faculty at Coastal Carolina University change throughout the year? Ultimately, it was found that there was no fluctuation in *S. aureus* phage nor in coliphage until one sample had a positive microbial test for coliphage in March. Despite the lack of fluctuations throughout the year, microbial and molecular phage detection techniques conducted this year have shown a drastic decrease in coliphage and *S. aureus* phage in comparison to past years. The second question asked in this study was can a correlation between the perceived stress level of the participant and bacteriophage presence be determined? There was no way to determine if there was a correlation between stress level and bacteriophage presence due to the drastic alterations in people's normal way of life, due to the COVID-19 pandemic. There has been an increase of stress level which could increase the presence of *Propionibacteria* causing competition for *E. coli* and *S. aureus* to thrive in the human microbiome. Consistent mask wearing also poses the potential for changes in the human microbiome, thus causing competition between other bacterial strains against *E. coli* and *S. aureus*, and indirectly decreasing the coliphage and *S. aureus* phage population. Human hygiene has altered in the past year as more people are hand washing and using hand sanitizer on a regular basis, which could eliminate bacterial strains such as *S. aureus* and *E. coli* on the human face and consequently their respective phage.

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Notes and References

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[1] Sulakvelidze A, Alavidze Z, Glenn Morris Jr. J. 2001. Bacteriophage Therapy. Antimicrob Agents Chemother. 45(3): 649–659.

[2] Keen E. 2015. A century of phage research: Bacteriophages and the shaping of modern biology. Bioessays. 37(1): 6–9.

[3] Campbell A. 2003. The future of bacteriophage biology. Nat Rev Genet. 4(6): 471–477.

[4] Lobanovska M, Pilla G. 2017. Penicillin's Discovery and Antibiotic Resistance: Lessons for the Future? Yale J Biol Med. 90(1): 135–145.

[5] Klein E, Smith D, Laxminarayan R. 2007. Hospitalizations and Deaths Caused by Methicillin-Resistant Staphylococcus aureus, United States, 1999 –2005. Emerg Infect Dis. 13(12): 1840–1846.

[6] Pal S. 2014. Trends in MRSA Prevalence. US Pharm. 2014; (39):6.

[7] Kourtis AP, Hatfield K, Baggs J, et al. 2019. Epidemiology and Recent Trends in Methicillin-Resistant and in Methicillin-Susceptible Staphylococcus aureus Bloodstream Infections — United States. Vital Signs 68:214–219.

[8] DeLeo FR, and Chambers HF. 2009. Reemergence of antibiotic resistant Staphylococcus aureus in the genomics era. The Journal of Clinical Investigation 119(9):2464-2474

[9] David MZ, Daum RS. 2010. Community-Associated Methicillin-Resistant Staphylococcus aureus: Epidemiology and Clinical Consequences of an Emerging Epidemic. Clin Microbiol Rev. 23(3): 616–687.

[10] Kaur DC, Chate SS. 2015. Study of Antibiotic Resistance Pattern in Methicillin Resistant Staphylococcus Aureus with Special Reference to Newer Antibiotic. J Glob Infect Dis. 7(2): 78–84.

[11] Watford S, Warrington SJ. 2021. Bacterial DNA Mutations. Treasure Island (FL): StatPearls Publishing. [cited 7 June 2021]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459274/

[12] Pieterse L, Powers A, Pride D, van Onselen L, Leonne GE, Richardson PE. 2018. Investigating the Lytic *Staphylococcus aureus* Bacteriophage Reservoir Amongst a South Carolina University Population: Discovery, Characterization, and Identification of a Potential Bacteriophage Treatment for Methicillin-Resistant *Staphylococcus aureus*. 16 (1): 29-35.

[13] Dhabhar FS. 2009. Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection and immunopathology. Neuroimmunomodulation. 16(5):300-17

[14] Lee HS. 2009. Somatic coliphage families as potential indicators of enteric viruses in water and methods for their detection. PhD thesis. University of North Carolina Chapel Hill, Chapel Hill, NC, United States.

[15] Pantůček R, et al. 2004. Identification of bacteriophage types and their carriage in Staphylococcus aureus. Archives of Virology 149 (9):1689-1703.

[16] Cannon JF, Thurn NA, and Richardson PE. 2013. The effects of salinity, pH, temperature, and dissolved oxygen on sensitivity of PCR identification of T4 bacteriophage. Journal of the South Carolina Academy of Science 11 (2):17-20.

[17] Zari S, Alrahmani D. 2017. The association between stress and acne among female medical students in Jeddah, Saudi Arabia. Clin Cosmet Investig Dermatol. 10: 503–506.

[18] Platsidaki E, Dessinioti C. 2018. Recent advances in understanding Propionibacterium acnes (Cutibacterium acnes) in acne.