ABSTRACT

Title of Thesis: ADDRESSING ANTIMICROBIAL RESISTANCE: *ALOE VERA'S* POTENTIAL FOR BACTERIAL INHIBITION AND DERMAL FIBROBLAST PROLIFERATION

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Antimicrobial resistance has been an emerging global issue over the last several decades. Acquired resistance renders antimicrobial agents useless, with a recent report projecting ten

million deaths by 2050 caused by drug-resistant infections. In response, research output on biomedical and public health solutions to AMR has significantly increased, including investigations on active compounds in medicinal plants. *Aloe vera* is known for anti-inflammatory, antibacterial, and cell proliferative properties stemming from its anthraquinones, flavonoids, and polysaccharides. In this review, Team Aloesporin applied qualitative and quantitative techniques to characterize the state of AMR awareness and discuss *Aloe vera*'s capacity for serving as an antimicrobial, wound-healing agent. First, a public opinion survey was distributed at the University of Maryland, College Park to assess community knowledge of antimicrobial resistance and related practices. *Aloe vera*'s potency was then investigated through a minimum inhibitory concentration assay with *Staphylococcus aureus* and *Staphylococcus epidermidis*. Lastly, a cell proliferation assay was designed for dermal fibroblasts with 2.5% w/v *Aloe vera*, 100 nM methylene blue, and 100 nM bacitracin-supplemented media. Though the public opinions survey provided insight into the gaps in knowledge surrounding antimicrobial resistance and consumer practices, the preliminary bacterial and dermal fibroblast assays yielded inconclusive results regarding *Aloe vera'*s respective antibacterial and proliferative effects. This research suggests a need for further investigation of the optimal state and concentration of *Aloe vera* for wound-healing and effective antimicrobial stewardship to address the escalating issue of antimicrobial resistance.

ADDRESSING ANTIMICROBIAL RESISTANCE: *ALOE VERA'S* POTENTIAL FOR BACTERIAL INHIBITION AND DERMAL FIBROBLAST PROLIFERATION

by

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Thesis submitted in partial fulfillment of the requirements of the Gemstone Honors Program,
University of Maryland, College Park, 2024

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Preface

Microbes can develop resistance to antimicrobial medications over long-term and wide-spread use. This phenomenon is termed antimicrobial resistance (AMR) which is a pressing global issue that demands attention from the scientific community. It is sustained through a complex network of evolutionary mechanisms in microbes and societal uses of the same drugs, exemplifying the need for novel antimicrobial agents. Though substantial research has been conducted across the scientific community to overcome the drivers and mechanisms of AMR, drug development efforts continue to be outpaced by its progression among pathogens. In response, numerous public health campaigns aimed at protecting existing antimicrobial agents have become valuable tools for limiting AMR spread. As many studies have demonstrated AMR's potential for adverse health outcomes, deficiencies in knowledge and novel drug developments curtail the successful prevention and treatment of various illnesses. To protect valuable antimicrobial medicines, measures aimed at minimizing resistance must be considered.

Naturally-derived medicines played a critical role in developing modern antibiotics. Continuous research has been devoted to extracting active compounds from natural sources such as *Aloe vera*, a succulent plant with rich medicinal history. This review explores the modern landscape of antimicrobial resistance, how human practices contribute to its expansion, and *A. vera*'s application as a bacteriostatic – bactericidal and skin cell proliferative tool. The research presented was collected through the utilization of three methodologies: a public opinions survey, a bacterial assay design, and a cell proliferation assay design. The public opinions survey was formulated to assess general knowledge regarding antimicrobial resistance at the University of Maryland, College Park. In light of literature seeking to understand gaps in health practices that

drive antimicrobial resistance, Team Aloesporin incorporated this survey to gain insight on current public knowledge while designing protocols to study the effects of *A. vera* application. Both assays outline viable methodologies that can be pursued to understand the impact of *A. vera* on bacterial inhibition and cell proliferation.

Though experiments aimed at developing novel solutions are valuable for overcoming antimicrobial-resistant pathogens, infrastructure to protect these discoveries must exist to ensure their longevity. While *A. vera* and other medicinal plants can provide researchers with viable and sustainable drug development pathways, researchers can advocate for better use practices that will uphold the integrity of antimicrobial advancements. Throughout this thesis, Team Aloesporin addresses why continuing investigations on antimicrobial resistance is critical and why novel development strategies that focus on natural products, like *A. vera*, are valuable avenues for research.

This work was conducted by Team Aloesporin, an interdisciplinary team of undergraduate student researchers within the Gemstone Honors Program at the University of Maryland, College Park. The Gemstone Honors Program encourages honors students to engage with the scientific process beyond the context of a classroom.

Acknowledgements

We would like to acknowledge the Gemstone Honors Program at the University of Maryland, College Park for the opportunity to participate in a four-year multidisciplinary research program, and thank Dr. David Lovell, Dr. Allison Lansverk, Ms. Leslie Lizama, and Ms. Brianna Lucas for their support. In addition, we would like to extend our gratitude towards our team librarian, Ms. Judy Markowitz, for her guidance while beginning our research. Our team would like to give a special thanks to Dr. Amy Sapkota and her lab members, Dr. Leena Malayil and Dr. Suhana Chattopadhyay, for graciously helping us with our bacterial experiments, and to Ms. Elizabeth Izydore for her assistance with our dermal fibroblast experimental design. Additionally, our team would like to thank our panelists, Dr. David Straney, Ms. Jasmin El Kordi, and Ms. Heather Teitelbaum for participating in our senior thesis defense, with a special thank you to our panelist Dr. Joseph Rom for his advice on the bacterial assay. Finally, we would like to thank our mentor Dr. Kan Cao for her guidance and encouragement throughout our time in Gemstone.

Table of Contents

Preface……ii

Acknowledgements……iv

Table of Contents……...……v

List of Tables……vii

List of Figures……..……viii

List of Abbreviations……x

Chapter 1: An Overview of Antimicrobial Resistance

Section 1.1: Antimicrobial Resistance……..……...…1

Section 1.2: The Development of Resistant Pathogens…….……..3

Section 1.3: Consumer and Patient Behavior Analysis…….……..5

Section 1.4: Antimicrobial Agent Production and Distribution Trends……..7

Section 1.5: Current Research and Treatments for Antimicrobial Resistance……9

Section 1.6: Antimicrobial Stewardship……....11

Chapter 2: *Aloe vera* as an Alternative Avenue

Section 2.1: History of medicinal *Aloe vera*……13

Section 2.2: Bacterial Inhibition: Mechanisms Behind Active Compounds in *Aloe vera*……16

Section 2.3: Bacterial Inhibition: Studies Exploring *Aloe vera* Application……20

Section 2.4: Common Uses of the Antibiotic Bacitracin for Bacterial Inhibition……22

Section 2.5: Epithelial Proliferation: Mechanisms Behind Active Compounds in *Aloe vera*.. 23

Section 2.6: Epithelial Proliferation: Studies Exploring Direct *Aloe vera* Application……25

Chapter 3: Public Awareness Regarding Antimicrobial Resistance at the University of Maryland

Section 3.1: Overview of the Public Opinions Survey……29

Section 3.2: Methodology……30

Section 3.3: Results……33

Section 3.4: Discussion……40

Chapter 4: Understanding the Effectiveness of *Aloe vera* on Bacterial Inhibition

Section 4.1: Background of Bacteria……46

Section 4.2: Overview of the Minimum Inhibitory Concentration Assay……47

Section 4.3: Methodology……48

Section 4.4: Preliminary Results……50

Section 4.5: Discussion……54

Chapter 5: Understanding the Role of *Aloe vera* on Dermal Fibroblast Proliferation

Section 5.1: Overview of the Dermal Fibroblast Proliferation Preliminary Assay……..……57

Section 5.2: Methodology……58

Section 5.3: Preliminary Results……60

Section 5.4: Discussion……62

Chapter 6: Conclusion

Section 6.1: Conclusion……66

Section 6.2: Note to Future Undergraduate Students……68

Bibliography……69

List of Tables

Table 2.1: Overview of biologically active compounds in *A. vera* and associated functions

Table 3.1: Summary of public opinions survey question and answer choices

Table 5.1: Two-way ANOVA analysis with treatment groups and day as different variables

List of Figures

Figure 1.1: Defense mechanisms of microbes against antimicrobials

Figure 1.2: Methods of horizontal gene transfer

Figure 1.3: The five main objectives to address AMR as developed by the WHO

Figure 3.1: Frequency of response rate for antibiotic prescription accessibility

Figure 3.2: Frequency of Responses on public understanding of antibiotic resistance

Figure 3.3: Frequency of responses assessing concern of antibiotic misuse and abuse

Figure 3.4: Frequency of responses on the importance of overprescription on antibiotic resistance

Figure 3.5: Frequency of responses on the contribution of incorrect consumption on antibiotic resistance

Figure 3.6: Frequency of responses regarding the role of overconsumption on antibiotic resistance

Figure 3.7: Frequency of antibiotic use at UMCP

Figure 3.8: Antibiotic consumption practices at UMCP

Figure 4.1: 96 Well-plate set-up for preliminary MIC assay

Figure 4.2: Bacitracin MIC assay against *Staphylococcus aureus* and *Staphylococcus epidermidis*

Figure 4.3: Day 3 photograph of bacitracin MIC assay against *Staphylococcus aureus* and *Staphylococcus epidermidis*

Figure 4.4: Aloe vera MIC assay against *Staphylococcus aureus* and *Staphylococcus epidermidis*

Figure 4.5: Day 3 photograph of Aloe vera MIC assay against *Staphylococcus aureus* and *Staphylococcus epidermidis*

Figure 5.1: 12-well plate Schema for Four Day Experiment for DF proliferation assay

Figure 5.2: Mean cell count of DF in four treatment groups over the course of four days

List of Abbreviations

AMR: Antimicrobial resistance

HGT: Horizontal gene transfer

WHO: World Health Organization

SES: Socioeconomic status

LI: Low-income

HI: High-income

R&D: Research and development

GAP: Global Action Plan

NAP: National Action Plan

MIC: Minimum inhibitory concentration

MBC: Minimum bactericidal concentration

MRSE: Methicillin resistant *staphylococcus epidermidis*

MRSA: Methicillin resistant *staphylococcus aureus*

UMCP: University of Maryland, College Park

UMD: University of Maryland

DF: Dermal Fibroblasts

MB: Methylene Blue

ANOVA: Analysis of variance

Chapter 1: An Overview of Antimicrobial Resistance

Section 1.1: Antimicrobial Resistance

Antimicrobial resistance (AMR) is an evolutionary event that occurs when microbes develop resistance to antimicrobial agents designed to prevent their growth (WHO, 2023). Microbes utilize several defense mechanisms to acquire this ability, such as developing physical barriers to restrict drug entry, changing cellular machinery to exclude the antimicrobial target, or employing cytosolic enzymes to decrease treatment longevity within a cell (Figure 1.1) (CDC, 2022). Bacterial resistance is of particular concern due to its clinical consequences because it can negate the effects of treatments for common diseases like typhoid fever and cancer (Abushaheen et al., 2020). Another notable example of the effects of AMR is the reduced efficacy of the antimicrobial agent penicillin (Abushaheen et al., 2020). In the early 20th century, scientist Alexander Fleming accidentally discovered that the fungus *Penicillium notatum* inhibited the growth of bacteria by producing a beta-lactam molecule now known as penicillin (Lobanovska & Pilla, 2017). After years of extensive research and purification, penicillins became an overused, mainstream treatment for various infections; consequently increasing resistance in each new generation of drugs and the evolution of "superbugs." (Lobanovska & Pilla, 2017).

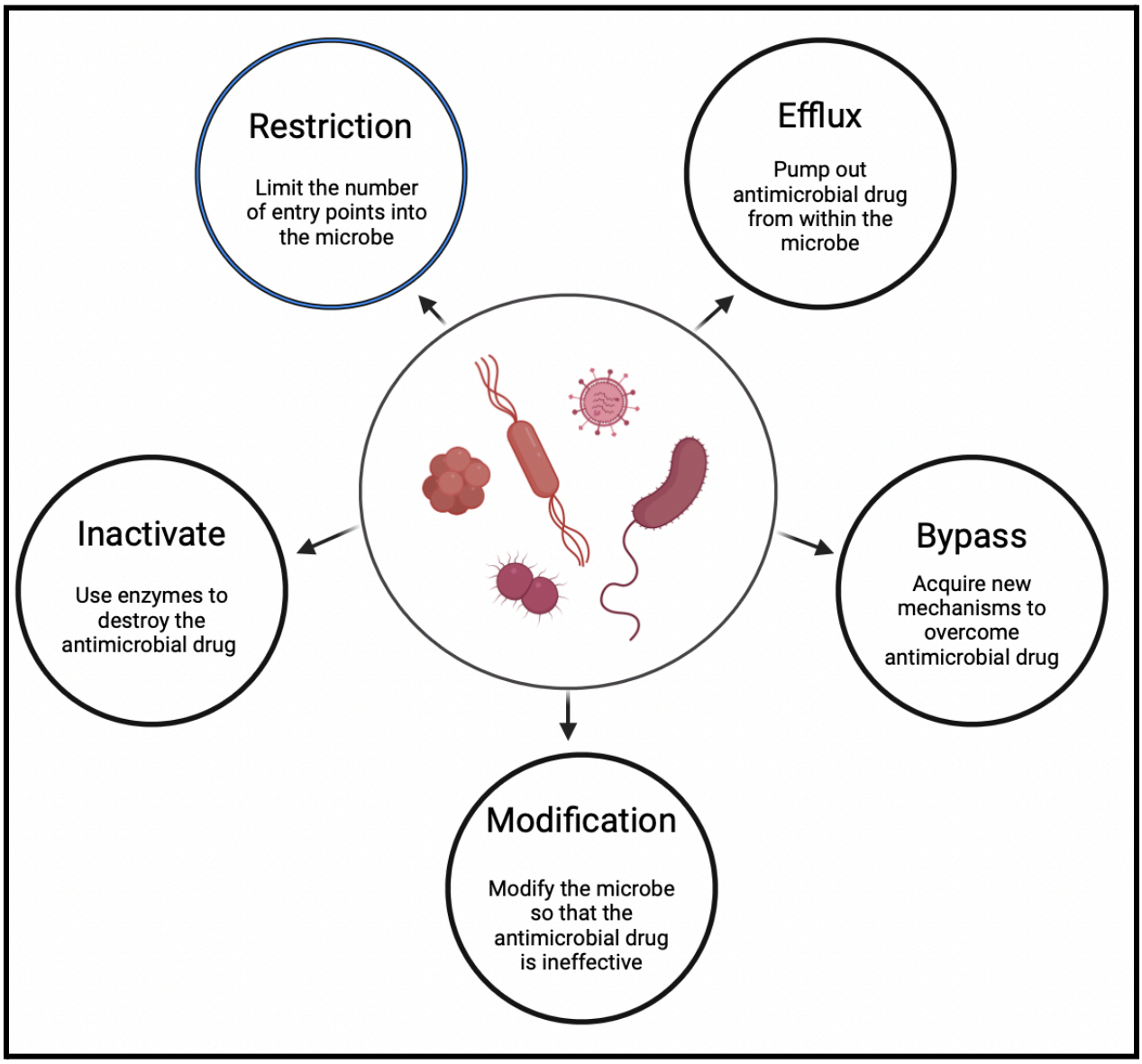

Figure 1.1: *Defense mechanisms of microbes against antimicrobials (CDC, 2022)*

Acquired resistance against penicillins over time, and its disastrous effects, display AMR's capacity to render traditionally successful treatments useless – leaving millions of people prone to deadly diseases (Abushaheen et al., 2020; Lobanovska & Pilla, 2017). A 2016 review approximated that 10 million deaths will occur annually due to AMR by 2050, with an associated economic burden of $100 trillion incurred by productivity loss. (O'Neill & Review on

Antimicrobial Resistance, 2016, p. 1). These AMR repercussions are projected to rise from more healthcare treatments and costs incurred by prolonged illnesses and hospital stays on an individual and global level, particularly within vulnerable populations (Dadgostar, 2019). Social disparities amplify the burden of AMR on marginalized populations due to limited access to quality healthcare, low awareness and education on the topic, and other influencing social determinants of health (Adebisi & Ogunkola, 2023).

Section 1.2: The Development of Resistant Pathogens

AMR is the evolutionary product of natural and acquired properties of microbes. Intrinsic natural resistance is an inherent "trait" of microbes that transpired from the interactions between antimicrobial-producing microorganisms (Reygaert, 2018). Acquired resistance is a phenomenon driven by horizontal gene transfer (HGT) pathways (Figure 1.2) or random mutations due to their relatively rapid rate of reproduction compared to the host (Reygaert, 2018). When placed under human-induced selective pressure, some microbes with acquired characteristics can survive even in the presence of antimicrobial agents (Reygaert, 2018; NIAID, 2011).

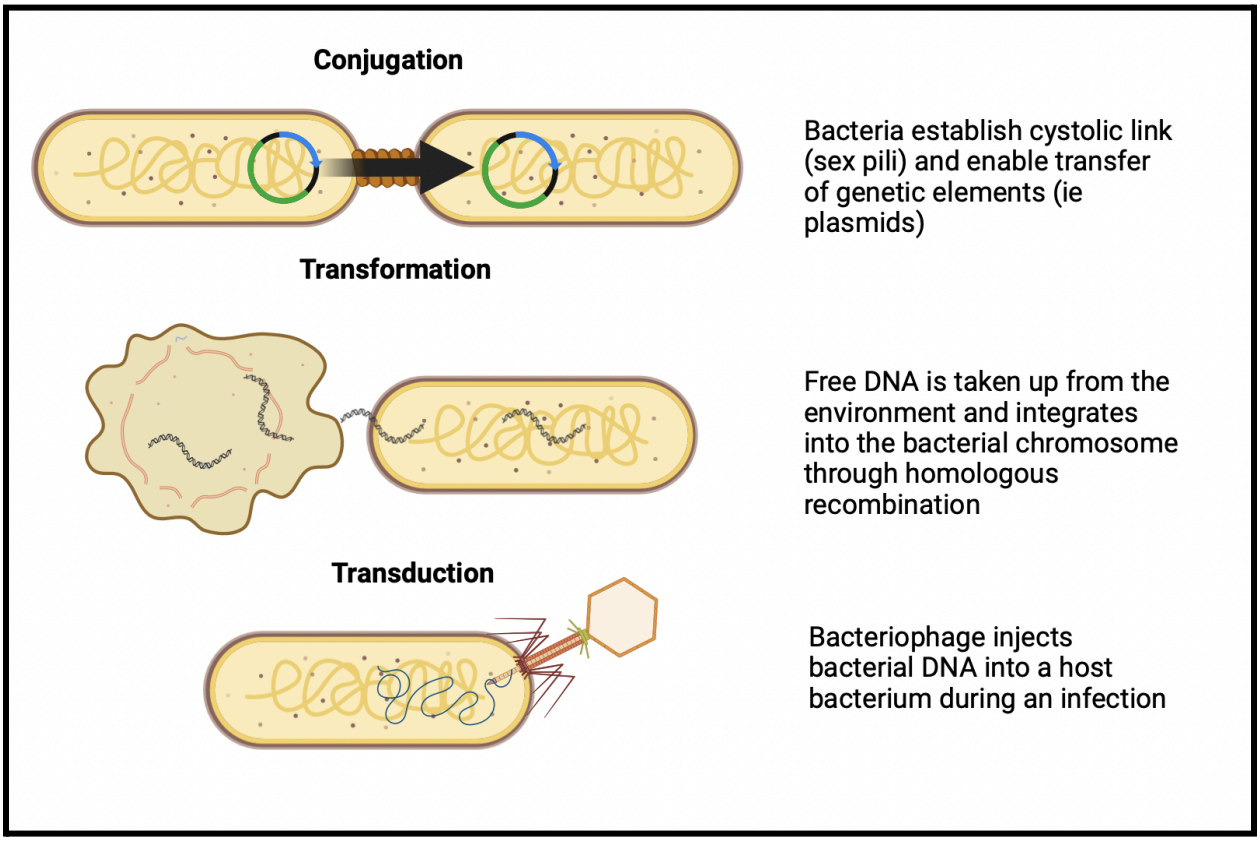

Figure 1.2 *Methods of horizontal gene transfer (Tankeshwar, 2024)*

AMR is also influenced by several societal factors such as antimicrobial usage within human health, agricultural applications, and treatment for domestic animals (Tang et al., 2023). Specifically, within the public health sector, the inappropriate usage and prescription of antimicrobial agents are major societal drivers for AMR growth (NIAID, 2011). Improper usage of antimicrobial drugs can take the form of not finishing a course of treatment, off-schedule consumption, and use for unrelated medical conditions (Wong et al., 2021). Moreover, prescription practices such as easy drug access, pharmaceutical motivation, and generalized treatment due to inadequate testing contribute to microbes' acquisition of AMR (Sweileh, 2021).

The failure of novel drug development exacerbates the development of resistance. Despite its fundamental role in healthcare, a 2023 progress report by the World Health Organization (WHO) and Global AMR R&D Hub listed only 77 antibacterial agents that were in development, and that many would fail to become available to consumers. Moreover, since a majority of these developing antibacterial agents belong to existing classes of antibiotics with established AMR pathways, they are placed at a higher risk for decreased long-term efficacy. An additional obstacle to novel antimicrobial development is its cost: few companies are interested in funding projects to produce and market antibiotics due to low return on investment (WHO & Global AMR R&D Hub, n.d., p. 2). In general, the rapid rate of resistance coupled with the stagnant development of novel medication is of international concern, and several identified risk factors are attributed to this *silent pandemic* (Tang et al., 2023).

Section 1.3: Consumer and Patient Behavior Analysis

Consumer-driven antimicrobial abuse is characterized by the improper administration or application of prescribed medication (Mason et al., 2018; WHO, 2023). Misuse stems from inadequate knowledge influenced by factors such as low socioeconomic status (SES) (Mason et al., 2018). One survey administered in the United Kingdom (UK) identified significant differences in some areas of AMR knowledge based on SES, defined as “affluent” or “deprived” (Mason et al., 2018). Regarding “prudent antibiotics usage,” 99% of “affluent” respondents claimed to always follow prescription instructions – an important practice in AMR prevention – and 85% responded they never stop treatment courses despite improved symptoms, whereas “deprived” respondents answered only 58% and 34%, respectively (Mason et al., 2018). Although knowledge and awareness is said to be associated with an understanding of AMR, misconceptions about AMR propagation exist throughout all levels of SES (Frost et al., 2023;

Mason et al., 2018). For instance, Mason et al. indicated that 43% of "affluent" respondents compared to 68% of "deprived" respondents were aware that antibiotics, commonly prescribed for bacterial infections, do not treat fungal infections, but only 46% and 55% of the respective populations knew that antibiotics were ineffective against viruses (Mason et al., 2018). Awareness is described to be the basis of AMR prevention, and it is clear that public understanding requires significant improvement despite SES (Mason et al., 2018). Misunderstandings that lead to excessive self-medication are propagated by the "germs are germs" perception adopted by many consumers with inflexible work or family schedules in search of immediate relief (Blaser et al., 2021, p. 5). Additionally, patients who may not understand the difference between distinct causative agents of disease may opt for unnecessary medication. For example, they may finish a bottle of previously untouched antibiotics, mirroring the attitude of their practitioners who believe that it couldn't "hurt" to take antibiotics (Blaser et al., 2021, p. 5).

Discussions about AMR are incomplete without considering its impact on communities without access to quality healthcare. When antimicrobial treatments become less effective due to consumer misuse and overuse, those living in poverty are likely to be hit the hardest, lacking resources to adapt effectively (Frost et al., 2023, pp. 1-3). Novel therapeutics, sanitation facilities, and healthcare professionals are often not available to communities in low-income (LI) and middle-income (MI) countries, leaving them disproportionately susceptible to AMR infections (Frost et al., 2023, pp. 3-4). Without infrastructure to control infection spread through sanitation and health practices, communities become reliant on subpar and broad-spectrum and over-the-counter (OTC) antimicrobial medicines (Blaser et al., 2021, p. 11; Frost et al., 2023, p. 3). Although these drugs are effective against a variety of pathogens, using narrow-spectrum

antibiotics would minimize side-effects and risk of AMR (Basu & Garg, 2018, pp. 2-3). Individuals in LI and MI countries that are suffering drug shortages are more likely to be overprescribed second-choice antibiotics by healthcare professionals, leading to increased AMR development (Frost et al., 2023, p. 1; Blaser et al., 2021, p. 11). Public health campaigns advocating for clean water access, stronger infrastructure, and increased healthcare quality have tremendous potential to raise awareness of AMR and limit the spread of superbugs, particularly in impoverished communities (Frost et al., 2023, p. 3).

Section 1.4: Antimicrobial Agent Production and Distribution Trends

Beyond the general public, there are multiple stakeholders that are affected by the development of AMR and the production of new antimicrobials. Key figures that shape AMR trajectory and response include but are not limited to: the pharmaceutical industry, healthcare sector, health-based international organizations, and national governments (Nambiar & Asha, 2023). The pharmaceutical industry is a widely recognized producer of antimicrobial drugs through innovative research and quality control (Nambiar & Asha, 2023). Despite this role, pharmaceutical companies are hesitant to invest in the development of novel antimicrobials, including antibiotics, primarily due to the cost of drug development, testing, and approval, which was estimated in 2017 to be around $1.5 billion (Plackett, 2020). Additionally, government policies maintain a low cost for drugs, while practitioners limit the distribution of medication, yielding lower profits for antibiotic production (Plackett, 2020). Thus, it is a common occurrence that companies are downsizing on antibiotic research and development (R&D) to pursue more profitable drug production, such as therapeutic treatments for chronic conditions, which can value around $1 billion in net present value (Abushaheen et al., 2020; Plackett, 2020).

As previously mentioned, the public health sector can influence the growth of AMR and is split into three broad fields: primary care, tertiary care, and detection (Frost et al, 2023, p. 1; Nambiar & Asha, 2023). Especially in primary care settings, providers are responsible for writing prescriptions but face a paradoxical situation: limit the distribution of antimicrobial drugs or allow people to suffer due to inadequate access to medications (Frost et al., 2023, p. 1). This behavior disproportionately impacts underserved populations who cannot afford expensive labs and testing, and therefore providers have to make broad judgements based on clinical observations and prescribe medications (Basu & Garg, 2018, p. 2). Further links to prescription practices include the difference between narrow and broad antibiotic treatments; broad-antibiotics may be prescribed more often because they are correlated with strength, but this habit will build more AMR in the future (Basu & Garg, 2018, pp. 2-3).

International organizations are responsible for the global response to AMR and guide national government policies through guidelines and goal-setting (Nambiar & Asha, 2023). The *Global Action Plan* (GAP) developed by the WHO in 2015 outlines five key components to tackle AMR to ensure safe use of antimicrobial drugs to treat and prevent infection diseases (Figure 1.3**)** (WHO, 2015, pp. 5-6). Based on the GAP outline, nations develop their own national action plans (NAP) and strategies to implement global objectives to combat AMR; since 2020, 120 countries developed a NAP (Naing et al., 2021). Despite national efforts in the past decade, public policy is labeled as inadequate mostly because of the lack of awareness and urgency on the part of policy-makers (Naing et al., 2021). In addition, an in-depth analysis of 108 NAPs suggests that many of these plans lack detail regarding financial support and resource allocation and thus present obvious gaps in policy-making (Charani et al., 2023).

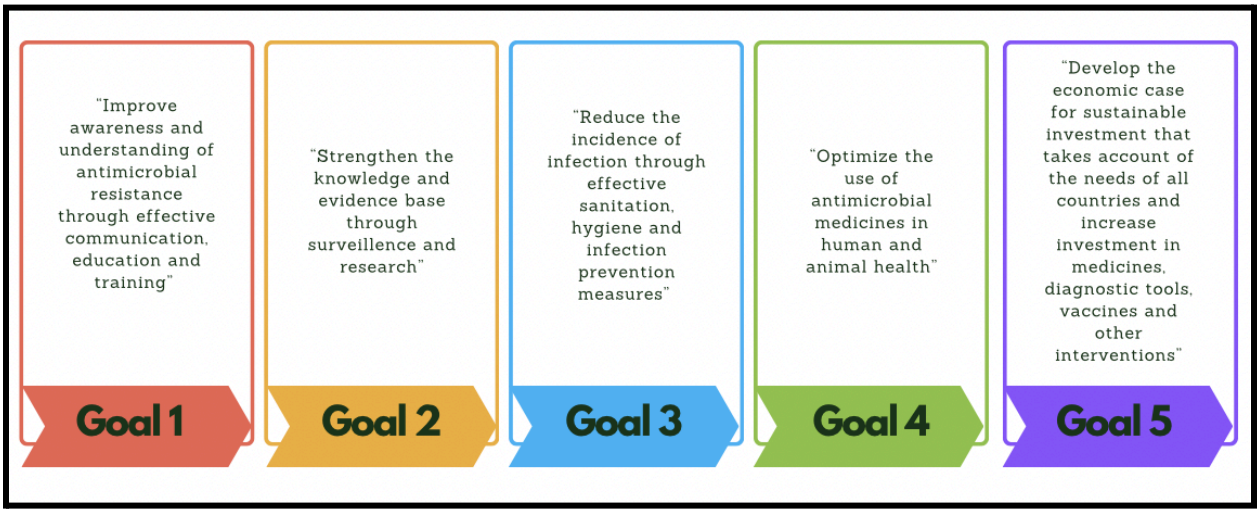

Figure 1.3 *The five main objectives to address AMR as developed by the WHO (WHO, 2015)*

Section 1.5: Current Research and Treatments for Antimicrobial Resistance

How researchers have responded to the threat of AMR burden has varied with time and geographical location. An interactive user interface developed by Luz et al. offers a wealth of information on publication trends by geographical region (Luz et al., 2022, p. 3). From 1999 to 2018, research output concerning AMR increased by 450%, but novel drug development did not follow the same trend (Luz et al., 2022; Tang et al., 2023). Subtopics within AMR research such as "Strategies for emerging resistances and disease," were frequently investigated around the world over the twenty-year period, though investigations regarding "Stewardship," and "Nanoparticles" emerged as popular topics by 2018 (Luz et al., 2022). International trends, in particular, show "Active compound extraction from plants" as the sixth-most researched subtopic in the world with 3,582 publications, compared to only 97 publications created in the United States within the same time frame according to the user interface (Luz et al., 2022, p. 3).

Alongside synthetic antimicrobials like methicillin or vancomycin which are traditionally associated with the term antibiotic, natural products have played a crucial historical role in drug discovery (Atanasov et al., 2021; Nandhini et al., 2022). Many of the therapies understood as antibiotics today are derived from naturally-produced compounds in fungi or bacteria, a pertinent example being penicillin (Mason et al., 2018). Though the United States has historically dedicated less AMR research to active compound extraction from natural sources than other countries, research on natural compound potential has increased in the past several years (Atanasov et al., 2021; Luz et al., 2022). To some, natural products will serve as the groundwork for synergistic therapies and synthetic development that will transform the field of antimicrobial production and help overcome AMR (Atanasov et al., 2021; Miethke et al., 2021).

In light of increased AMR research exploring the relationship between AMR and stewardship, numerous strategies have been implemented to protect existing antimicrobial treatments. In 2005, the WHO began categorizing antimicrobial agents based on disease severity, the existing number of therapies for the disease, and the potential pathways of transmission, including AMR gene acquisition and spreading from non-human infections (Wendlandt et al., 2015). These classifications prevent prescribers from mis- or overusing critically important agents (CIA) such as lipopeptides, a membrane-binding bactericidal antibiotic recognized for its ability to treat *methicillin resistant Staphylococcus aureus* (MRSA), while streamlining research efforts by drawing attention to treatment areas that require further innovation (Nandhini et al., 2022; Wendlandt et al., 2015). Recent classification developments include AWaRE: Access, Watch, and Reserve (Essential Medicines et al., 2021). This system prioritizes treatments from lowest to highest resistance potential while considering the effect of certain antibiotic classes on AMR and uses explicit language to describe whether they should be readily used to treat

infection (Essential Medicines et al., 2021). Additionally, medications belonging to the "Reserve" category have proven applications against WHO-identified critical or priority pathogens; by reserving their use, researchers hope to slow the development of AMR and keep crucial medications effective for those who need them (Essential Medicines et al., 2021).

Section 1.6: Antimicrobial Stewardship

Antimicrobial stewardship (AMS) is a multifaceted term that describes the strategies that enable the proper use of antimicrobial agents (Doron & Davidson, 2011; Dyar et al., 2017). While there is a significant emphasis on the management of antimicrobials on human health, the concept of stewardship also includes efforts within a comprehensive 'one health' approach (Dyar et al., 2017). As previously described, there are many ways that world actors can contribute to the spread of AMR (Nambiar & Asha, 2023).

There are three major areas of efforts that define AMS. First, healthcare workers and practitioners must focus on controlling the appropriate prescription of antimicrobial medicines to contain the accessibility of these valuable resources, and appropriately treat various infections. Secondly, prevention strategies must be optimized to address the misuse and overuse of antimicrobial drugs. Finally, the overarching goal of AMS is to hinder the rapid progress of AMR and challenge rising morbidity and mortality rates (Doron & Davidson, 2011). There are several strategies to address these broad goals of AMS, including but not limited to prior authorizations for prescriptions, therapeutic guidelines, and general education of the stakeholders and the general public (Doran & Davidson, 2011). In particular, the WHO emphasizes public awareness and education as core components to address AMR because a lack of knowledge correlates to the increased spread of resistance (Wong et al., 2021). Understanding the

significance of completing antibiotic treatment courses as prescribed and only when prescribed is crucial for limiting patient-driven AMR (Mason et al., 2018). Public health campaigns such as ‘World Antibiotic Awareness Week’ and ‘Keep Antibiotics Working’ have been organized to improve antibiotic prescription and usage guidelines through social media outreach and communications marketing (Gilham et al., 2023; Iwu et al., 2020).

Chapter 2: *Aloe vera* as an Alternative Avenue

Section 2.1: History of Medicinal Aloe vera

A. vera is a species of plant that belongs to the Xanthorrhoeacae family and is widely popularized for its medicinal properties (Martinez-Burgos et al., 2022). The historical use of *A. vera* dates back to ancient Mesopotamia and Egypt, where the medicinal properties of the plant were recorded on clay tablets and Ebers Papyrus respectively. Particularly in ancient Egypt, historians report it as a staple compound in the "beauty regimes" of the Queens Nefertiti and Cleopatra (Mehta, 2017, p. 22). The plant was also famed in ancient Greece and Rome, with the ancient Greeks chronicling its wound healing abilities and treatment for skin conditions, and tales of Alexander the Great conquering the island of Socotra for its *A. vera* reservoir to help his soldiers (Martinez-Burgos et., 2022; Mehta, 2017, p. 22). Additionally, it has been used in traditional Chinese, Japanese, and Indian medicine for over two thousand years (Radha et al., 2015). Hence, for thousands of years, *A. vera* was explored for its antioxidant, anti-inflammatory, and antiseptic properties (Sanchez-Machado et al., 2017; Surjushe et al., 2008).

Fast forward to modern times, the first method of extracting and stabilizing *A. vera* into a natural, active gel for commercial purposes was pioneered by Dr. Bill Coates in 1968 (Manvitha & Bidya, 2014, p. 86). Starting in the nineties, a significant increase in crop demand arose in response to the increasing awareness of its medicinal and nutritional capabilities. In fact, a report

in 2018 indicated that the market value of *A. vera* was approximately $1.60 billion and would only increase. Beyond its use in traditional medicine practices, *A. vera* extract can now be found in foods and common cosmetic products such as shampoos and lotions (Martinez-Burgos et al., 2022).

A. vera's chemical composition provides insight into its medical properties. Although there are many species within the *Aloe* genus, *Aloe barbadensis Miller* is the most cultivated given its bioactive profile and extensive research backing (Martinez-Burgos et al., 2022; Sanchez-Machado et al., 2017). The succulent plant is known for its high water content of over 99% and abundant compounds like anthraquinones, flavonoids, vitamins, enzymes, sterols, and sugars (Radha et al., 2015). Understanding whether *A. vera*'s active compounds possess antibacterial and skin cell proliferative properties, as well as the mechanisms of action, would provide insight on whether the plant would be an effective solution to address AMR and associated wound-healing (Table 2.1).

Compound	Specific Name	Function(s)	Source
Anthraquinones	Aloe Emodin	Changes in genes for peptidoglycan synthesis and biofilm formation Reduction in cell membrane permeability	(Li et al., 2021; Qun et al., 2023) (Li et al., 2021)
	Aloin	Promotes fibroblast, vascular endothelial, and keratinocyte proliferation through factors like EGF Increase fibroblast migration and tissue granulation	(Li et al., 2017; Shafaie et al., 2020) (Li et al., 2017)
Flavonoids	Flavonoids (general, glycone, aglycone)	Disrupt biofilm formation	(Andrade Lopes et al., 2017; Onsare & Arora et al., 2015)
	Dihydroxy flavonoids	Inhibit LasR/RhIR noncompetitively and reduce quorum-sensing capabilities	(Paczkowski et al, 2017)
	Quercetin	Reduce biofilm formation, polysaccharide intercellular adhesion, and bacterial hydrophobicity	(Mu et al., 2020)
Vitamins	Vitamin C	Inhibit bacterial growth of through oxidative stress and acidity	(Kallio et al., 2012)
	Vitamin E	Decrease biofilm production and surface adhesion in gram-positive bacteria Prevent nitric oxide production and lipid peroxidation in dermal fibroblasts Increase levels of wound-healing	(Vergalito et al., 2019) (Butt et al., 2017) (Butt et al., 2017)

		factors like VEGF or bFGF	
Enzymes	Carboxypeptidase	Can degrade N-formyl-met-leu-phe	(Li et al., 2002)
Sterols	Lupeol	Nitric oxide production related to lipid peroxidation, plasma membrane depolarization, and cell division arrest	(Kim & Lee, 2021)
Polysaccharides	Acemannan	Modulates cell-cycle progression by regulating translation of cyclin D1 as a result of the AKT/mTOR pathway	(Xing et al., 2015)
	Glucomannan		

Table 2.1 *Overview of biologically active compounds in A. vera and associated functions*

Section 2.2: Bacterial Inhibition: Mechanisms Behind Active Compounds in Aloe vera

There are several mechanisms, either bactericidal or bacteriostatic, by which compounds found in *A. vera* halt bacterial growth. Bactericidal compounds directly kill bacteria, while bacteriostatic compounds inhibit growth without necessarily killing the bacteria (Bernatová et al., 2013). Firstly, compounds may interfere with peptidoglycan synthesis which can inhibit bacterial growth (Li et al., 2021). Compounds may also prevent biofilm formation, which is when bacteria adhere to surfaces to create a matrix of extracellular polymeric substances that aid in their growth (Onsare & Arora, 2015; Andrade Lopes et al., 2017; Tizazu & Bekele, 2024; Mu et al., 2021; Vergalito et al., 2018). Another process that can halt bacterial growth is the inhibition of quorum sensing, the manner of communication and coordinated gene expression among bacteria to control virulence factor production (Paczkowski et al., 2017). Moreover,

interference with mRNA or protein synthesis can lead to either bacteriostatic or bactericidal effects, depending on the extent of disruption and the specific mechanism targeted (Li et al., 2021).

Anthraquinones are organic, polycyclic structures with known antibacterial activities (Qun et al., 2023). Specifically looking at aloe emodin, an anthraquinone found in *A. vera*, a review elucidated that the compound can influence peptidoglycan and biofilm synthesis affiliated genes (Martinez-Burgos et al., 2022; Qun et al., 2023). Additionally, aloe emodin's higher level polarity compared to other anthraquinones is asserted to have more potent antibacterial effects (Qun et al., 2023). In regards to bacteria associated with the epidermis, Li et al. tested aloe emodin against multidrug-resistant *Staphylococcus. epidermidis* and other species with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays. The results of the study found that for *S. epidermidis*, the MIC with aloe emodin ranged from 4 to 32 μg/mL and the MBC ranged from 32 to 128 μg/mL depending on the strain. At the MBC concentration 128 μg/mL, a time-kill assay determined that 4 hours after aloe emodin treatment, 96.9% of methicillin resistant *S. epidermidis (MRSE)* were eradicated (Li et al., 2021). Moreover, transcriptional profiles of *S. epidermidis* treated with aloe emodin found a reduction in gene transcripts associated with biofilm formation. Additionally, Li et al. indicated that aloe emodin at 4 μg/mL reduced the selective permeability of cell membranes for *S. epidermidis*. The researchers' proposed mechanism of cell membrane perturbation in gram-positive bacteria is credited to aloe emodin conformational binding to peptidoglycan (Li et al., 2021).

Flavonoids typically consist of two benzene rings linked by a three-carbon heterocyclic ring, with specific classes including flavones, flavonoids, flavonols, and flavan-3-ols, all of which are found in *A. vera* (Chen et al., 2023). A review by Tizazu & Bekele compared the

different flavonoid profiles between *Aloe* species, and found *A. vera* to contain flavonoids such as saponarin, naringenin, rutin, catechin, and epicatechin, corresponding to a high antioxidant capacity (Tizazu & Bekele, 2024). Onsare & Arora specifically studied antibiofilm impact of flavonoids derived from the the seeds of *Moringa oleifera* on three different bacterial genus. At the end of a 24 hour incubation, an MIC assay of flavonoids against biofilm disruption in *Pseudomonas aeruginosa* demonstrated 88% growth inhibition (Onsare & Arora, 2015). Paczkowski et al. built on these findings by examining the mechanism behind *P. aeruginosa* quorum-sensing inhibition through biochemical analyses and predictions. The authors determined that flavonoids, particularly with a dihydroxy A-backbone, noncompetitively prevent LasR/RhIR DNA binding (Paczkowski et al., 2017). In flavones specifically, two hydroxyl moieties in the A-ring backbone were determined to be key players in LasR/RhIR inhibition. Overall, the implications of flavonoid administration to inhibit quorum sensing in bacteria like *P. aeruginosa* is to reduce virulence and pathogenicity of microbes (Paczkowski et al., 2017). Bacteriostatic effects were also studied in high biofilm producing *S. aureus* strains. Andrade Lopes et al. conducted MIC assays that determined that most tested glycone and aglycone flavonoids were effective in inhibiting biofilm formation, despite not significantly impeding the growth of *S. aureus* strains (Andrade Lopes et al., 2017). Aglycone flavonoids were especially potent in inhibiting biofilm formation in *S. aureus* strains that overexpressed genes responsible for efflux proteins (Andrade Lopes et al., 2017). Quercetin, a flavonoid abundant in *A. vera*, was shown to hinder biofilm formation in *S. epidermidis* ATCC 35984 (Mu et al., 2021; Tizazu & Bekele, 2024). Results demonstrated that quercetin effectively reduced biofilm formation at varying concentrations, with over 95% inhibition at a 500 mg/mL. The influence of quercetin treatment on *S. epidermidis* is linked to a decreased hydrophobicity, reduced levels of certain

exopolysaccharides, and the downregulation of polysaccharide intercellular adhesion (Mu et al., 2021).

A. vera contains vitamins such as A, C, E, B12, and more (Hęś et al., 2019). Kallio et al. found that 5 mM of vitamin C fully inhibited *S. aureus* growth, but also exhibited modulatory behavior in conjunction with quercetin. The experiment showed that 90 μM quercetin with 1 mM of vitamin C reduced *S. aureus* growth to 3% of the control group , for which only 1mM vitamin C was used, highlighting its potent effects. The proposed mechanism of action is vitamin C is acidic, can increase oxidative stress on bacterial cells, and stabilize quercetin (Kallio et al., 2012). Vergalito et al. also tested the effect of vitamin E on the growth of *S. aureus*, *S. epidermidis*, and other species. In the presence of vitamin E, with or without varying concentrations of glucose, biofilm formation in both species was significantly reduced and vitamin E was shown to significantly reduce the *Staphylococcus* species capacity to colonize the surface of a medical device (Vergalito et al., 2018).

Enzymes found in *A. vera* include alkaline phosphatase, amylase, bradykinase, carboxypeptidase, catalase, cellulase, lipase, and peroxidase (Surjushe et al., 2008). Carboxypeptidase, a class of enzyme that degrades peptides from the C-terminal, was experimentally shown to have an inhibitory effect on bacterial growth in a bactericidal manner (Li et al., 2002; Sapio & Fricker, 2014). Li et al. conducted an assay on the percentage of inoculated *S. epidermidis* killed in the presence or absence of carboxypeptidase Y. The researchers found that carboxypeptidase Y degrades N-formyl-met-leu-phe, a peptide which can inhibit the death of *S. epidermidis* cells. In other words, the carboxypeptidase indirectly promoted the death of *S. epidermidis* cells by blocking an inhibitor of it (Li et al., 2002).

Nevertheless, limited research has been conducted on the effects of the other enzymes found in *A. vera* on the growth of specific bacterial strains.

A. vera also contains four sterols, one of which is lupeol (Surjushe et al., 2008). Kim & Lee determined that in *E. coli*, 5 µg/mL of lupeol induced nitric oxide production which is associated with lipid peroxidation and membrane depolarization. Moreover, lupeol induced nitric oxide was found to promote cell division arrest in *E. coli*, and bacterial cell death without DNA fragmentation. (Kim & Lee, 2021).

Section 2.3: Bacterial Inhibition: Studies Exploring Aloe vera Application

There is an abundance of literature that has indicated that *A. vera* is effective against many bacterial species. Arbab et al. tested *A. vera* extract created from the leaf or root macerated with either distilled water or ethanol (Arbab et al., 2021). The *A. vera* extracts were tested on *Shigella, Salmonella, E.coli* and *S. aureus* isolates from septic wounds on animals. The *A. vera* extracts were compared to ten antibiotics. The results indicated that all pathogens tested were susceptible to the antibacterial activity of 100 µL of the *A.vera* extracts with significant zones of inhibition (Arbab et al., 2021). The study indicated that both the gram-negative and gram-positive isolates were found to have the higher susceptibility to the ethanol leaf and root extracts with greater zones of inhibition ranging from 25 to 30%, whereas the non-ethanol *A. vera* leaf and root extract had zones of inhibition between 20 to 25% (Arbab et al., 2021). Some antibiotics had lower zones of inhibition when compared to the *A. vera* extracts, such as Ciproflaxin with *E. coli* at only 12% inhibition. The remaining conventional antibiotics tested had comparable results to the *A. vera* extracts, with none of them exceeding the inhibitory activity demonstrated by *A. vera*. Hence, Arbab et al. raised the possibility that *A. vera* could be

employed as an antibacterial agent to treat bacterial skin infections brought on by these specific pathogens with increased potential with the addition of ethanol (Arbab et al., 2021).

Additionally, Mehrishi et al. conducted experiments on methanolic extracts of three medicinal plant - *Azadirachta indica* (neem)*, A. vera,* and *Mentha piperita* (peppermint) - to determine the antibacterial and antibiofilm activity of the plants (Mehrishi et al., 2022). The extracts were tested on multidrug-resistant clinical isolates of *Staphylococcus saprophyticus, Acinetobacter baumannii, P. aeruginosa, S. aureus* and *S. epidermidis*. These bacterial species were chosen due to their multidrug-resistant status and classified by their biofilm production abilities: strong, moderate, or weak (Mehrishi et al., 2022). The researchers used an agar well diffusion to measure antibacterial activity, a MBC assay to determine the smallest concentration necessary for bactericidal activity, followed by a modified crystal violet assay to determine biofilm production. All three plant extracts were able to inhibit the bacteria to an extent, but *A. indica* and *A. vera* had higher antibacterial and antibiofilm activity compared to *M. piperita*. Overall, the study conducted by Mehrishi et al. highlights the possible use of *A. vera* for its antibacterial and antibiofilm properties against multidrug-resistant bacteria like *S. aureus* and *S. epidermidis* (Mehrishi et al., 2022).

Prior literature has also investigated the potential inhibitory properties of *A. vera* by utilizing MIC assays. Goudarzi et al. experimented with *A. vera* against 140 strains of *P. aeruginosa,* a highly resistant pathogen associated with wound infections. The study conducted the MIC assay by formulating a concentration of 100 g of *A. vera* gel to 1 liter of 2% DMSO. The results showed that most *P. aeruginosa* strains were inhibited by the *A. vera* gel extract at a MIC of 400 μg/mL or lower, with five strains (10.6% of the total) requiring a higher concentration of 800 μg/mL to achieve inhibition (Goudarzi et al., 2015). An additional study

conducted by Habibi et al. performed a MIC assay of 100 g *A. vera* to 250 mL distilled water against *Klebsiella pneumoniae, Serratia marcescens, E. coli, Salmonella enterica, Shigella dysenteriae,* and other species. They found the MIC for *E. coli* and *S. enterica* at 0.2 µg/ml (Habibi et al., 2018). Both studies were successful in determining the MIC of *A. vera* for various bacteria indicating the success of MIC assay for the investigation of *A. vera's* antimicrobial properties which can be used to specifically study *S. aureus* and *S. epidermidis*.

Section 2.4: Common Uses of the Antibiotic Bacitracin for Bacterial Inhibition

Although active compounds like flavonoids and anthraquinones are associated with bacterial inhibition, most topical cut-and scrape ointments are made of antibiotics like bacitracin, a cyclopeptide antibiotic originating from two species of *Bacillus* (Nguyen et al., 2022; Zhu et al., 2023). Bacitracin's broad-spectrum activity stems from its disruption of cell wall biosynthesis through the lipid II cycle, making it a popular antibiotic in topical ointments designed to treat minor injuries (Nguyen et al., 2022; Radeck et al., 2016; Zhu et al., 2023). Similar to other antimicrobials, bacitracin is a valuable health resource threatened by AMR. Given that community-spread *methicillin-resistant S. aureus* (MRSA) is becoming increasingly prevalent, many people turn to OTC ointments like bacitracin to treat infection. Unfortunately new strains of MRSA are becoming highly resistant to bacitracin, and through selective pressure grow stronger (Kim & Jeon, 2016). Additionally, many OTC ointments are meant to target infections but not promote wound-healing (Draelos et al., 2021).

The most influential driver of wound-healing is a clean and moist environment (Draelos et al., 2011). Historically, antibiotic ointments were applied to injuries to keep the area moist and prevent infections at high rates. More recently, it was discovered that there is no variation in

infection rates between applying an antibiotic ointment versus white petroleum (Draelos et al., 2011). Moreover, infection rates in healthcare settings have significantly improved, with dermatological postoperative infections only around 1.5%. Therefore, it is suggested that the use of topical bacitracin can be considered unnecessary without proper consideration (Draelos et al., 2011).

Furthermore, there is an emerging field of research surrounding topical ointments that actually induce skin cell proliferation, and hence wound-healing. For instance, there are pharmacological studies assessing the use of recombinant human epidermal growth factor (rhEGF) in topical ointments that can reduce wound closure time and scar formation in rats (Young-Bae et al., 2006). Research has also shifted towards exploring natural sources because of the various biological components that can extend beyond healing and serve a dual function with both antimicrobial properties and additional medical effects (Criollo-Mendoza et al., 2023). As mentioned previously, *A. vera* is widely popularized due to antimicrobial functions stemming from active ingredients like flavonoids and anthraquinones. Accordingly, understanding the dual role of *A. vera* as an antimicrobial and skin cell proliferative agent provides insight as to its potential for serving as an alternative to topical ointments and becoming a novel pathway to address AMR.

Section 2.5: Epithelial Proliferation: Mechanisms Behind Active Compounds in Aloe vera

The epidermis is the outermost layer of the skin and protects the body from harmful radiation, substances, and pathogens (Yousef et al., 2022). Underneath the epidermis is the dermal layer, which is fibrous connective tissue made of elastin and collagen that supports the skin. The most prevalent cell type found in the dermis are dermal fibroblast (DF) cells which

produce many of the extracellular matrix (ECM) substances crucial for wound healing (Brown & Krishnaumurthy, 2024; Cialdai et al., 2022). DF cells play a role throughout the healing process, specifically during the proliferative phase of wound healing as they mitigate re-epithelization by degrading the fibrin clot, producing essential ECM components like fibronectin, and formation of granulation tissue (Cialdai et al., 2022).

Studies reveal that compounds in *A. vera* such as aloin – an anthraquinone – and polysaccharides, like acemannan and glucomannan, can contribute to wound-healing n (Shafaie et al., 2020, p. 235). Aloin is linked to endothelial and fibroblast cell proliferation and can regulate epidermal growth factor (EGF) (Shafaie et al., 2020, p. 235). One study examined the effect of aloin on wound-healing for medicinal purposes. The results of Li et al. indicated that at concentrations of 125 μg/mL and 250 μg/mL, there was an increase in human skin fibroblast proliferation; similar trends were seen with immortalized keratinocytes and endothelial cells. Additionally, an aloin solution was observed to increase wound-healing, measured by how long it took to close a wound, in rats at a low and high doses (Li et al., 2017). This could be tied to aloin's potential to increase migration of human skin fibroblasts. An *in vitro* scratch test revealed that at varying concentrations of Aloin, from 7.8 μg/mL to 125 μg/mL, significantly higher migratory rates for the fibroblasts were present (Li et al., 2017).

Polysaccharides acemannan and glucomannan are also promoters of DF cell proliferation. Acemannan in particular is suggested to be associated with re-epithelialization on mice by activating the AKT/mTOR pathway. An *in vivo* investigation showed that injecting varying concentrations of acemannan near a surgically-induced wound on mice accelerated the healing process and increased levels of cyclin D1 (Xing et al., 2015). In addition, the study also demonstrated how the compound promotes primary fibroblast proliferation *in vitro* through the

upregulation of Cyclin D1 translation (Xing et al., 2015). A vital part of the prior literature is also focused on the impact of acemannan on gingival fibroblasts proliferation, and an examination revealed that DNA synthesis under acemannan conditions of 2 mg/mL to 16 mg/mL. The mechanism of the gingival fibroblast proliferation and healing is tied to the upregulation in factors like collagen I (Jettanacheawchankit et al., 2009, pp 527-530).

While Vitamin E in *A. vera* has been shown to aid in bacteriostatic mechanisms, it also has a vital role in protecting the epithelium. The compound has been incorporated up to a concentration of 1% in many OTC creams because it can prevent nitric oxide production or lipid peroxidation caused by UV light. In addition, vitamin E acts as a protective response to epidermal oxidative stress (Keen & Hassan, 2016). Butt et al. also found that pre-treatment of DF cells with vitamin E downregulated the expression BAX, a pro-apoptotic gene, in response to heat. The researchers also observed an increase in basic fibroblast growth factor (bFGF), EGF, and VEGF which all play a role in wound-healing (Butt et al., 2017).

Section 2.6: Epithelial proliferation: Studies exploring direct Aloe vera application

Multiple studies have also examined how the direct administration of *A. vera* gel is associated with wound healing due to its active compounds. Anggraeni & Roeslan studied how 10, 50, and 100 µg/mL concentrations of gel derived from *A. vera* would impact bFGF, protein synthesis, compared to *Clincanthus nutans*, in fibroblasts as observed by an ELISA assay. After 24 hours, all *A. vera* treatment groups had elevated bFGF levels (Anggraeni & Roselan, 2022, p. 21). The research suggests that given elevated levels of bFGF, a mitogenic factor with an important role in fibroblast proliferation, the application of *A. vera* gel may facilitate accelerated wound healing processes (Anggraeni & Roeslan, 2022, p.23). The mitogenic bFGF was also

studied in an *in vivo* experiment of rats with second degree burns. 30 mg/Kg of *A. vera* powder was dissolved in 1mL saline and topically applied on the rat burns over the course of 30 days. The authors examined higher levels of bFGF expression, along with elevated TGF-β1, VEGF, and Prdx6 expression in the *A. vera* group against the control group of just saline (Atiba et al., 2022). The elevated expression of various growth factors further suggests *A. vera* gel contains multifaceted effects on wound healing, involving modulation of signaling pathways and biological processes, such as inflammation and angiogenesis, essential for tissue repair in burns (Atiba et al., 2022).

Other gene transcript changes have also been observed in *A. vera* treated fibroblasts and endothelial cells. Shafaie et al. conducted several evaluations, such as a real-time RT PCR and scratch test, using the supernatant of white pulp *A. vera* gel disinfected in chloroform (Shafaie et al., 2020, pp. 236-237). Results of the real-time RT PCR showed that in the 10% *A. vera* treated fibroblasts and endothelial cells, expression of integrin α1, β1, and PECAM-1 gene transcription was significantly increased (Shafaie et al., 2020, p. 240). Elevated gene transcription of integrins affiliated with the ECM suggests that *A. vera* gel may expand re-epithelialization and angiogenesis, potentially facilitating the formation of new blood vessels and improving tissue repair processes when applied on the epidermis (Shafaie et al., 2020, p. 242). Although elevated PECAM-1 gene transcripts were observed, flow cytometry revealed no significant difference in protein expression within 48 hours of treatment. This discrepancy may suggest the involvement of other regulatory factors that have yet to be investigated in the context of *A. vera* gel treatment (Shafaie et al., 2020, p. 243).

Despite abundant literature on the influence of *A. vera's* active components on wound-healing, there is vague information on the amount, or concentrate, and state of *A. vera*

used for optimal results. Commonly, many industries utilize the gel, or an extract, from the plant's leaves for pharmaceutical and cosmetic applications (Martinez-Burgos, 2022). As a brief overview of the different application methods, Rizqi & Fitriawan cultured fibroblasts with 5, 50, 125, 250, and 500 µg/mL *A. vera* gel extract. After 24 hours, fibroblast cell viability at 5 µg/mL of *A. vera* dried leaf powder in ethanol was significantly greater compared to the control, while cell migration was significantly increased from 5-250 µg/mL of treatment (Rizqi & Fitriawan, 2022, pp. 258-259). In another study, Teplicki et al. treated DF cells with 1%, 2% and 3% of *A. vera* liquid in a DMEM and 2% FBS media for a proliferation assay. Cell counts over the course of 5 days suggested that *A. vera* generally promotes fibroblast proliferation at concentrations of 2% and 3% (Teplicki et al., 2018). A different range of concentrations was used in a study where fibroblasts were treated with *A. vera* powder mixed with deionized water from 10 µg/mL to 320 µg/mL (Jose, 2020, p. 19). The fibroblasts were previously treated with 40 µM arecoline for its toxic potential to inhibit cell growth described in literature. Despite the toxic conditions, *A. vera* was able to significantly protect acroline-treated fibroblasts against cytotoxicity at EC50 75.091 µg/mL of *A. vera* (Jose, 2020, pp. 1-2). Unlike the Teplicki et al. and Rizqi & Fitriawan studies that looked solely at the promotive effects of *A. vera*, the Jose study explored its protective function. Another study observed the effects of *A. vera* on DF cell proliferation in the presence or absence of 30 ng/mL of FGF-2 on diabetic and nondiabetic cells. The *A. vera* extract percentages tested in the experiment were 0.6%, 1.25%, 2.5%, 5%, 10%, or 20% in cell culture media (Abdullah et al., 2003, p. 711). Based on this study, 20% *A. vera* was recorded as toxic, while 1.2% and 2.5% without FGF-2 increased the diabetic DF proliferation (Abdullah et al., 2003, pp. 713-714). Overall, these various studies show that there are still large gaps in literature regarding what an optimal concentration and state of *A. vera* is when conducting experiments *in*

vitro.

In the literature, *in vivo* experiments on rats have different methods of *A. vera* application. A 2021 in vivo study on rats with oral wounds was conducted in which the experimental groups were given a 300 mg/kg and a 500 mg/kg dose of *A. vera* gel daily, while the control group was given distilled water. Wound evaluation revealed increased epithelialization and fibroblast proliferation in the experimental groups (Rashid et al., 2021). Another study experimented the effects of *A. vera* gel extract on rat skin wounds with saline extract as the control. The results found that the mean number of fibroblasts in the *A. vera* group was significantly higher than the control group at almost 1.8 times the number of cells observed. However, no information was provided about the concentration of *A. vera* used (Girsang et al., 2020). Moreover, in a 2021 study, Meza-Valle et al. formulated an *A. vera* hydrogel with 1,2-propanediol (propanediol) and triethanolamine (TEA) and applied it onto 0.5cm^2 wounds on rats. Researchers observed a 29% reduction in total healing time within the *A. vera* treatment group compared to the control (Meza-Valle et al., 2021). Overall, although the concentrations and application methods of *A. vera* vary among the in vivo studies, multiple researchers demonstrate the positive impact of *A. vera* on cell proliferation.

Chapter 3: Public Awareness Regarding Antimicrobial Resistance at the University of Maryland

Section 3.1: Overview of the Public Opinions Survey

Despite variances in wealth, educational opportunities, and the implementation of awareness campaigns, a pervasive deficiency in accurate AMR knowledge persists across the global community, indicating an urgent need for innovative solutions beyond current initiatives. Understanding the local landscape of AMR awareness at the University of Maryland, College Park (UMCP), is imperative for developing targeted interventions and fostering informed decision-making among the community. By examining AMR awareness within this specific community, we can gain insights into the unique factors influencing knowledge and perceptions about AMR.

AMR within UMCP can be contextualized through acknowledging its broader impact on global public health. In the United States, a misconception persists that antibiotics are effective against viral infections, with roughly 25% of the population believing antibiotics are suitable for the common cold (Huh et al., 2018). Similarly, 19.6 to 25.6% of sample respondents from a survey conducted in Nigeria believed that a course of antibiotics could be prematurely completed without consulting a healthcare professional (Huh et al., 2018). Incomplete understanding of antibiotic use contributes to AMR by posing significant risks, including insufficient eradication of bacteria, spread of resistant strains, prolonged infections, and increased healthcare burden (WHO, 2023). Although self-medication is the target of many public awareness campaigns, public dissonance about the causes and implications of AMR, such as individuals' lack of understanding about their contributions to AMR, undermines their effectiveness (Fletcher-Miles

& Gammon, 2020). These discoveries illustrate the serious risk AMR poses to personal and public health, and emphasize the need for more thorough awareness and education measures.

By framing targeted questions based on a preliminary literature review of AMR, this survey aimed to reveal potential connections between consumer practices and knowledge regarding antibiotic use. Through highlighting the knowledge gaps observed in prior research, it sought to contribute valuable insights about AMR awareness at UMCP while contributing to the broader discussion on AMR as a critical public health issue.

Section 3.2: Methodology

To understand the necessity of AMR awareness and assess general knowledge, a public opinions survey was administered to students, faculty, and staff at UMCP via a Qualtrics form. The survey and distribution materials were submitted for ethical consideration to the IRB review board at the University of Maryland which were exempt from review based on federal IRB regulations and guidelines that outline exemption categories (Exemption Number: #45CFR46.104(d)(2)(ii)). The survey itself was created through Qualtrics with questions structured so respondents could select multiple choice answers, rate on a numerical likert scale, or submit short answers (Table 3.1). The questions based on a likert scale ranged from 1 to 10 where 1 represented answers such as “not well,” “not concerning,” or “unimportant” and 10 as the opposite end of the spectrum. Questions measuring participants’ understanding and knowledge on the basis of a 10-point likert scale were divided into four categories: 1-3 indicates poor understanding or knowledge, 4-5 indicates poor to moderate, 6-7 indicates moderate to proficient, and 8-10 indicates proficient.

	Question	Response Choices
1	Are you a student or faculty member?	UMD Student
		UMD Staff & Faculty Members
		Other
2	How accessible are antibiotic prescriptions to you?	I have full access
		I have partial access
		I have little to no access
3	On a scale of 1 to 10, with 1 being not well and 10 being very well, how well do you understand antibiotic resistance?	1 through 10
4	On a scale of 1 to 10, with 1 being not concerning and 10 being concerning, how concerning is the misuse and abuse of antibiotics in terms of bacterial resistance?	1 through 10
5	On a scale of 1 to 10, with 1 being unimportant and 10 being very important, how important are the following contributors to antibiotic resistance? a. The overprescription of antibiotics by medical professionals b. The incorrect consumption of antibiotics c. The overconsumption of antibiotics	1 through 10
6	Please select antibiotic as your response for this question:	Prescription
		Bacteria

		Antibiotic
		Resistance
7	How often do you use antibiotics?	Rarely
		Sometimes
		Frequently
		Every Day
8	What do you do when you have a cut or scrape?	Free Response
9	When you use antibiotics, do you take them as prescribed?	I never take antibiotics
		I don't finish the full course of treatment; I stop taking them when I feel better.
		I take all of them and as prescribed
10	Please provide your email address if you would like to be entered in the gift card raffle.	Free Response

Table 3.1: *Summary of public opinions survey question and answer choices*

The questions of the survey assessed participants' opinions regarding: accessibility to antibiotics, understanding of antibiotic resistance, the concern regarding misuse/abuse of antibiotics and how it leads to bacterial resistance. These questions connect to the importance of overprescription, incorrect consumption, overconsumption, frequency of usage of antibiotics, what participants do in a cut or scrape scenario, and whether they take antibiotics as prescribed.

A question to check attention was added to the survey to compensate for any variability based on respondents to account for discrepancies in sample size (Table 3.1).

The survey was distributed across UMCPs through flyers with a QR code to the Qualtrics form and could also be accessed virtually via a URL link. The flyers were posted in buildings across campus, and the URL link was sent to campus organizations and classrooms to disseminate the information. These were the main materials used and distributed. As an incentive for completing the survey, participants were provided an opportunity to receive either a $10, $15, or $25 digital Visa gift card. Award recipients were chosen among respondents who provided email information (n = 115). Python-based analysis facilitated the random selection of three respondents using the index of their assignment within the new dataframe.

Section 3.3: Results

A total of 179 responses were collected from September 2023 to February 2024. After filtering the responses to exclude non-UMCP students, invalid responses, and the singular facility response, there were a total of 144 valid responses that were used for data analysis. With regards to the question 2, “how accessible are antibiotic prescriptions to you,” findings revealed varied levels of access among respondents. Out of the 144 participants, 113 claimed full access to antibiotic prescriptions, representing 78.47% of the sample size. Additionally, 24 respondents reported having partial access, accounting for 16.67% of the sample, while only 7 participants (4.86% of the total) claimed to have no access to antibiotic prescriptions (Figure 3.1).

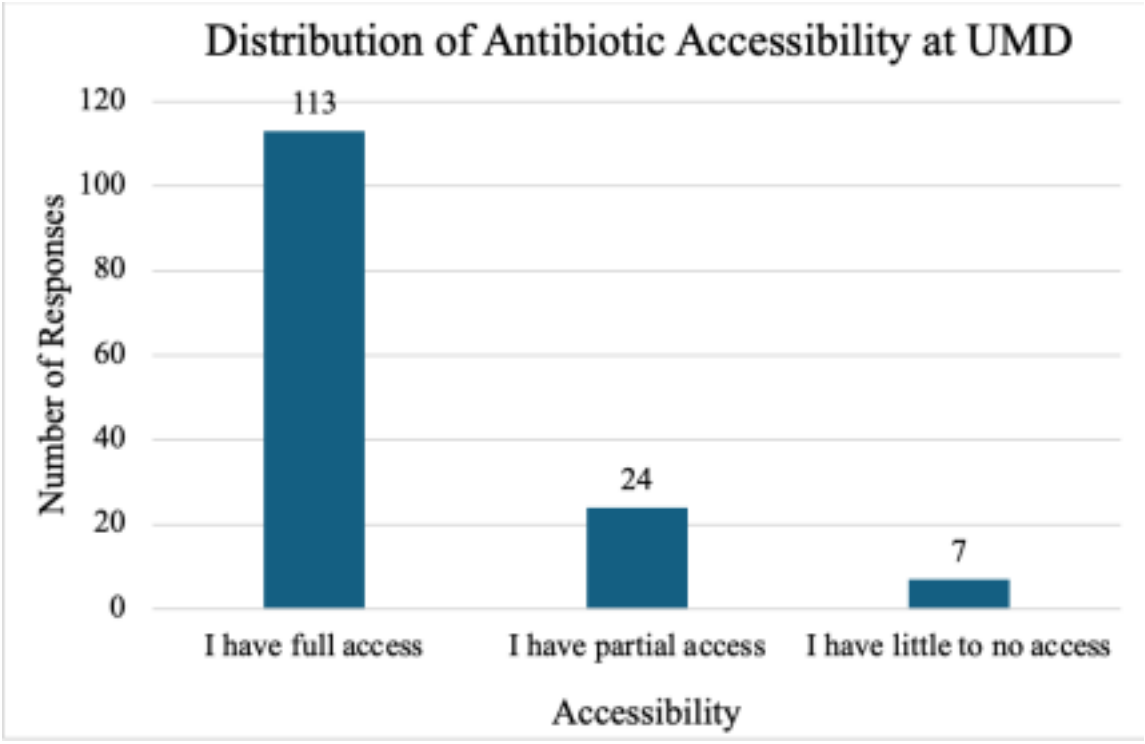

Figure 3.1: *Frequency of response rate for antibiotic prescription accessibility*

The following question 3 aimed to gauge the respondents' understanding of antibiotic resistance based on a numerical likert scale, with 1 being not well and 10 being very well. The dataset showcased a somewhat left-skewed distribution, with a sample mean of 6.44, a median of 7, and variance of 5.79. Notably, a significant proportion of respondents, approximately 61.11% of the sample size rated their understanding level at 7 or above (Figure 3.2).

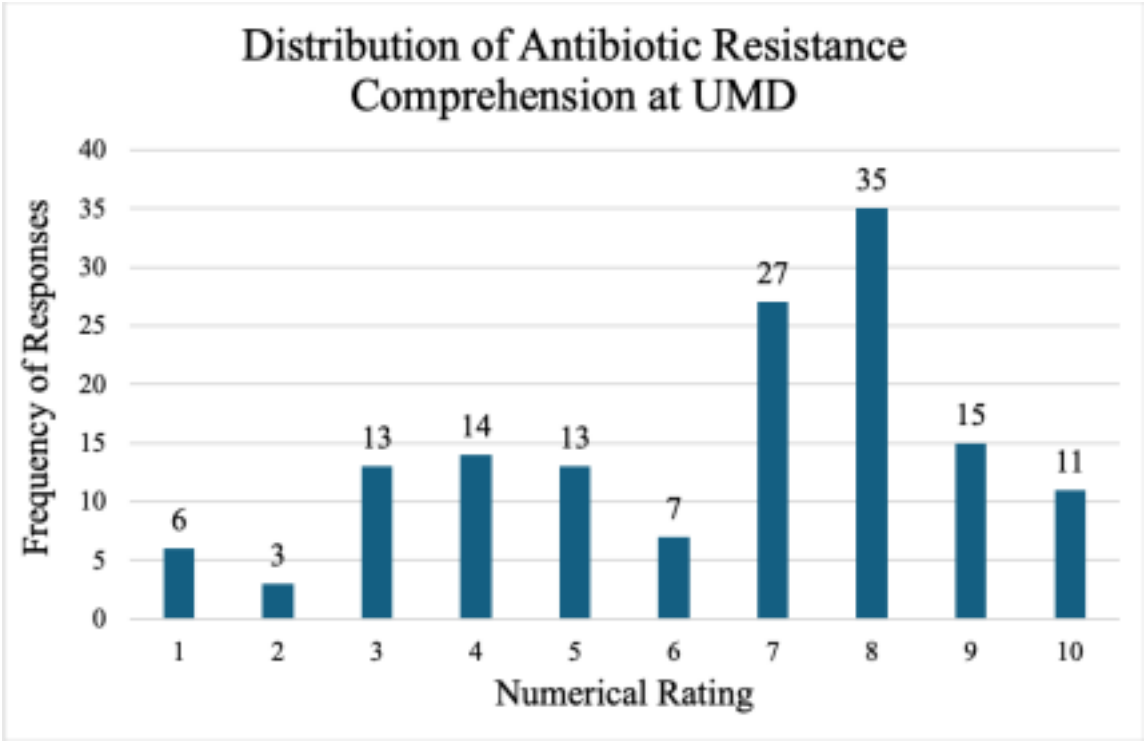

Figure 3.2 *Frequency of Responses on public understanding of antibiotic resistance*

Similarly, question 4 assessed participants' level of concern about antibiotic misuse and abuse in relation to bacterial resistance with a numerical likert scale, with 1 signifying not concerning and 10 signifying very concerning. The sample mean response for this question was 6.77, with a median of 7, and the variance computed to be 6.18. The distribution of responses is left-skewed, with approximately 60.84% of respondents in the sample size rating their level of concern at 7 or above. (Figure 3.3).

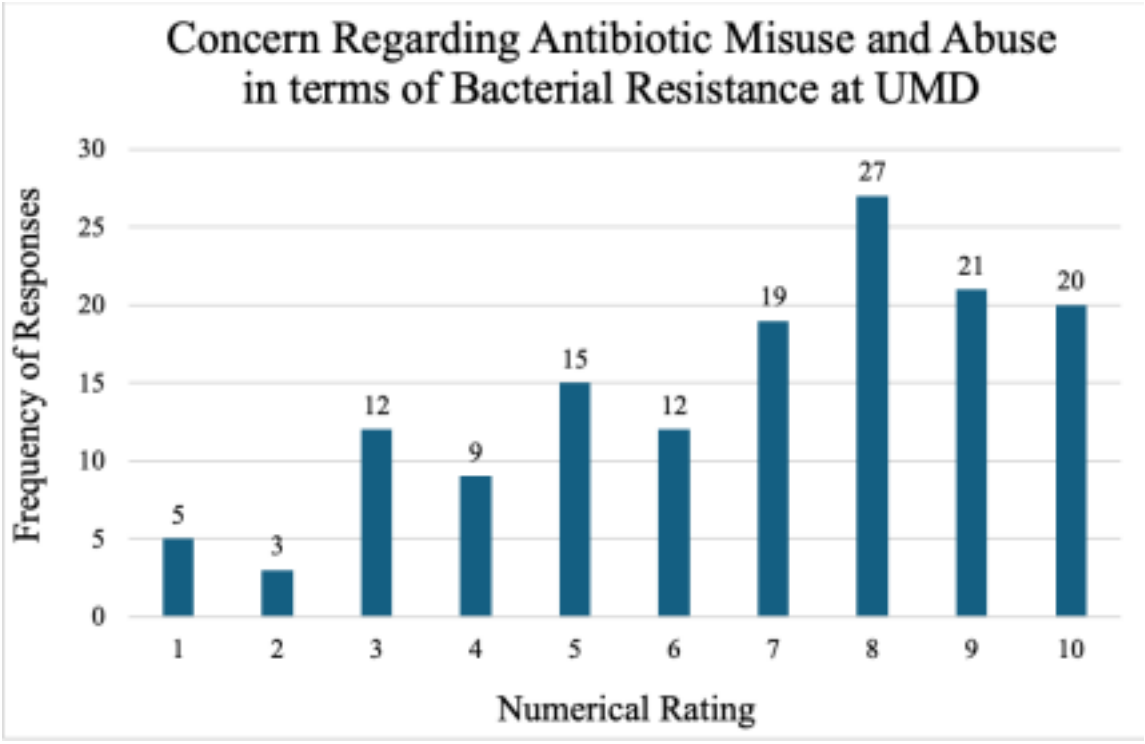

Figure 3.3 *Frequency of responses assessing concern of antibiotic misuse and abuse*

The next part of the survey, question 5, revealed the perceived importance of overprescription of antibiotics with a numerical likert scale, with 1 indicating unimportant and 10 as very important. The graph was strongly left-skewed with a sample mean of 7.79, a median of 8, and a variance of 3.68 (Figure 3.4). A follow-up rating asked respondents about how much they believe incorrect consumption contributes to antibiotic resistance. The data in Figure 3.5 indicates that the frequency of the response is reverse J-shaped with a sample mean of 7.56, with a median of 8, and a variance of 5.18. Finally, the last rating in this question measured the participants' perceptions regarding the importance of overconsumption of antibiotics on increased resistance (Figure 3.6). Based on the numerical likert scale, the sample mean of the data is 8, with a median of 9, and a variance of 3.94, while the response distribution is also displayed as reverse J-shaped. For each of these ratings, approximately 84.02%, 72.53%, and 81.12% of respondents in the sample size rated the importance at a level 7 or above for parts a,

b, and c respectively, as 7 or above on the Likert scale.

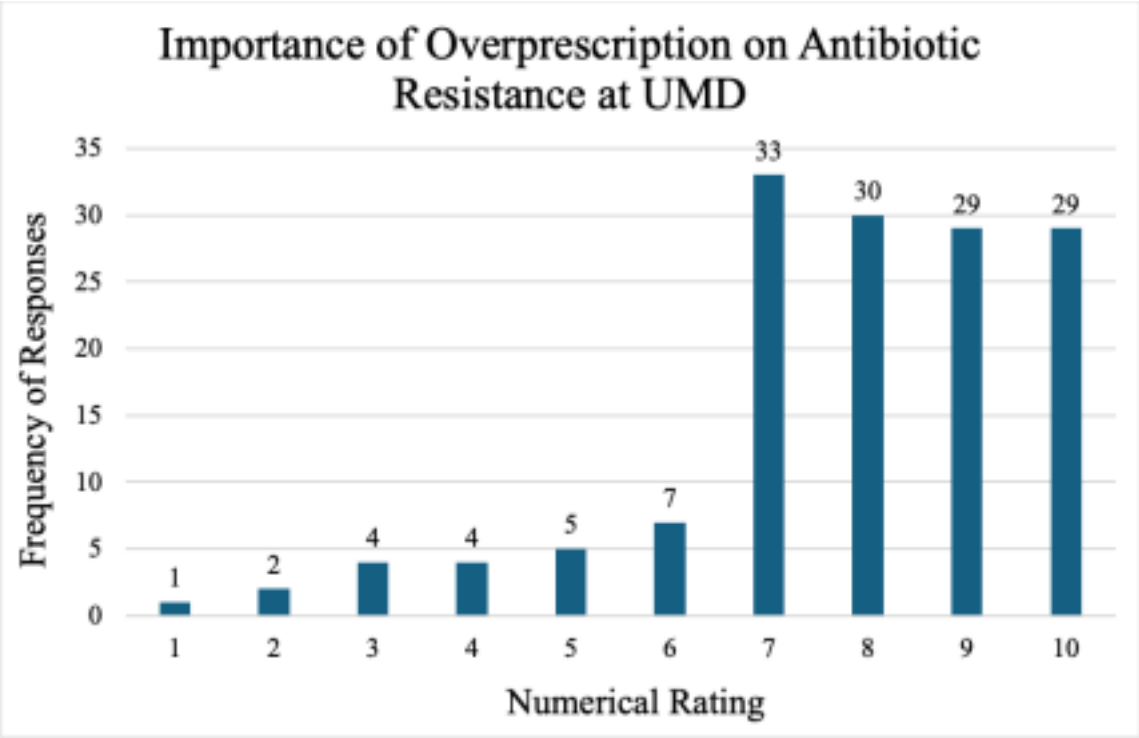

Figure 3.4: *Frequency of responses on the importance of overprescription on antibiotic resistance*

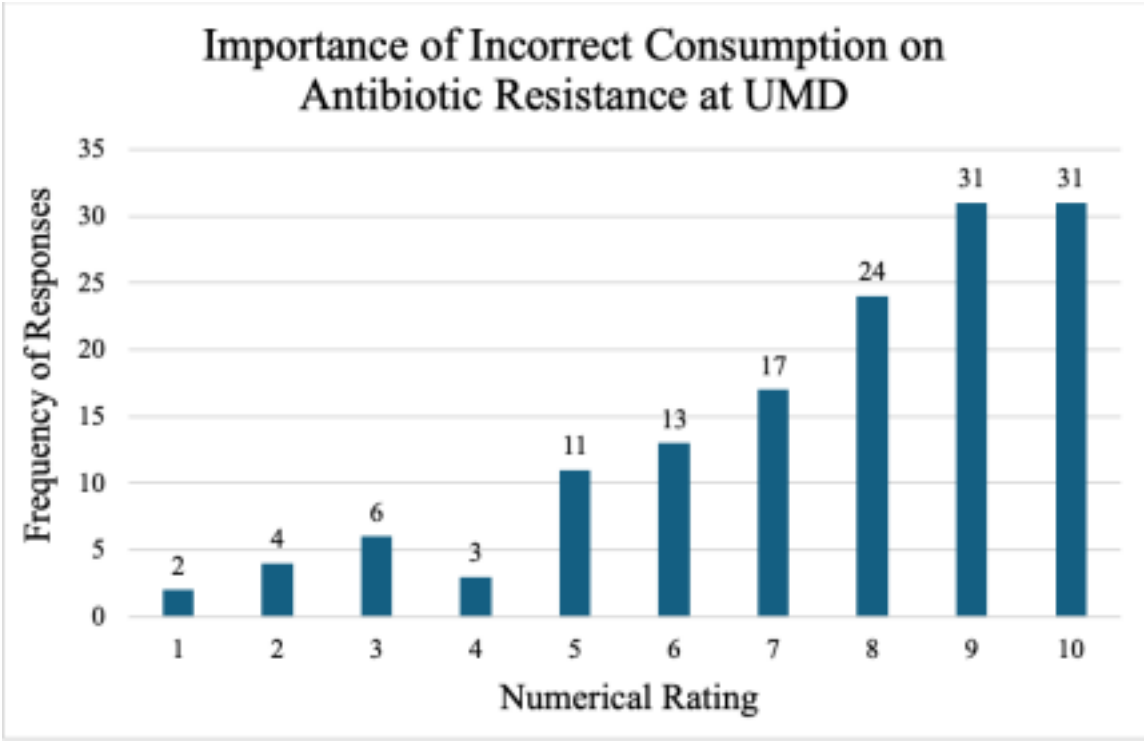

Figure 3.5: *Frequency of responses on the contribution of incorrect consumption on antibiotic resistance*

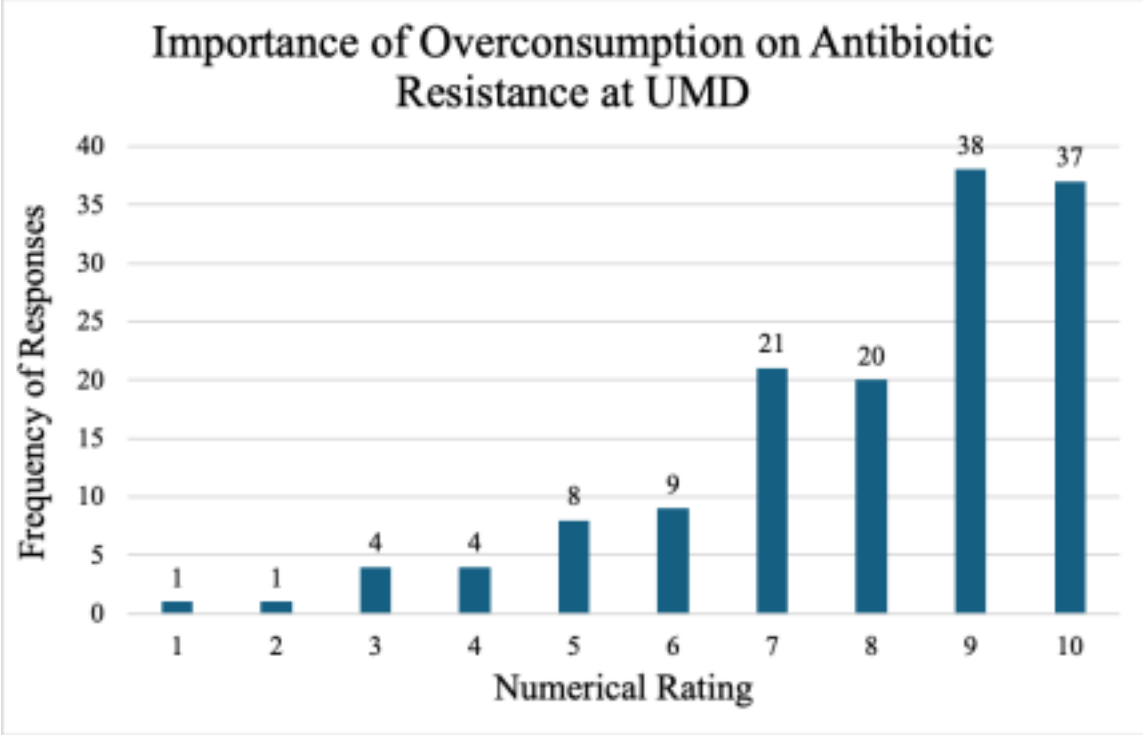

Figure 3.6: *Frequency of responses regarding the role of overconsumption on antibiotic resistance*

Aside from understanding respondents' awareness regarding the global phenomenon of AMR, the following questions 7 and 8 attempted to perceive consumer practices. Figure 3.7 displays the frequency of responses in regards to how often respondents use antibiotics. The data presents itself as J-shaped with 61.11% of the respondents in the sample size answering “rarely.” Additionally, 34.72% of respondents in the sample size stated that they use antibiotics sometimes, while a small percentage reported frequent (3.47%) or daily (0.69%) antibiotic use. A similarly formatted question assessed how long respondents stayed on a course of treatment if they do have to take antibiotics. Based on the data displayed, the majority in the sample size, approximately 73.61%, reported “I take all of them as prescribed” (Figure 3.8). In contrast, 18.06% of respondents admitted to not completing the full treatment, opting to stop taking antibiotics when they felt better. Additionally, 8.33% of respondents indicated that they never take antibiotics.

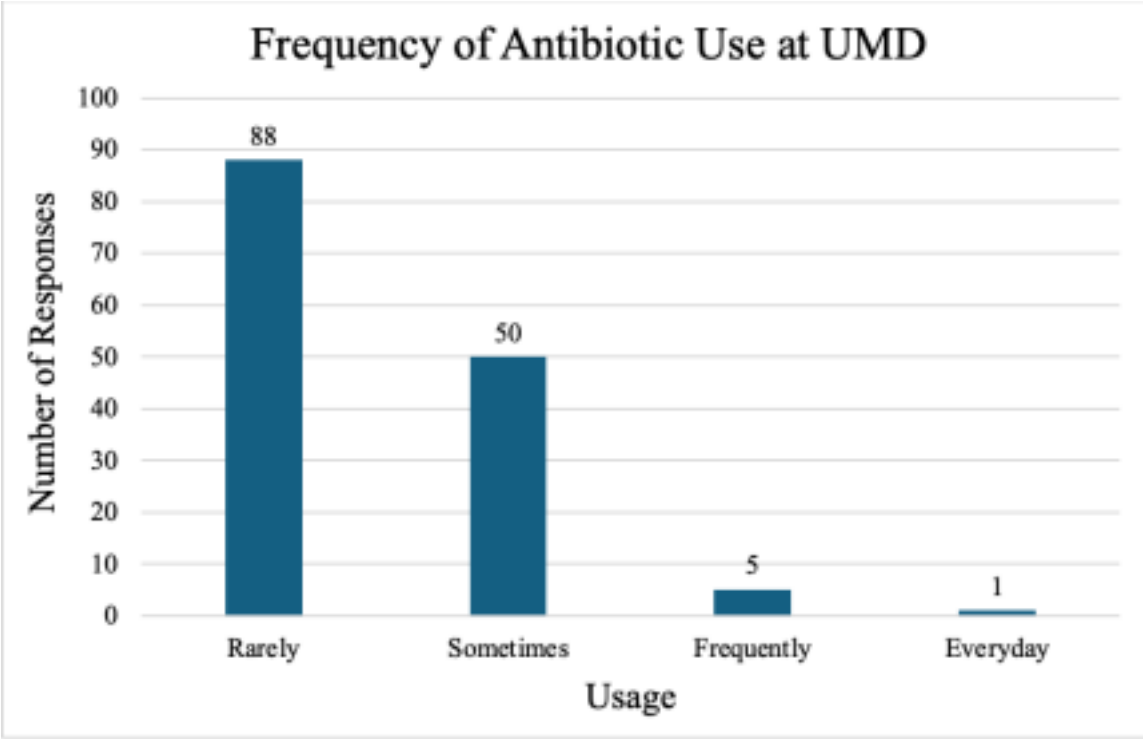

Figure 3.7: *Frequency of antibiotic use at UMCP*

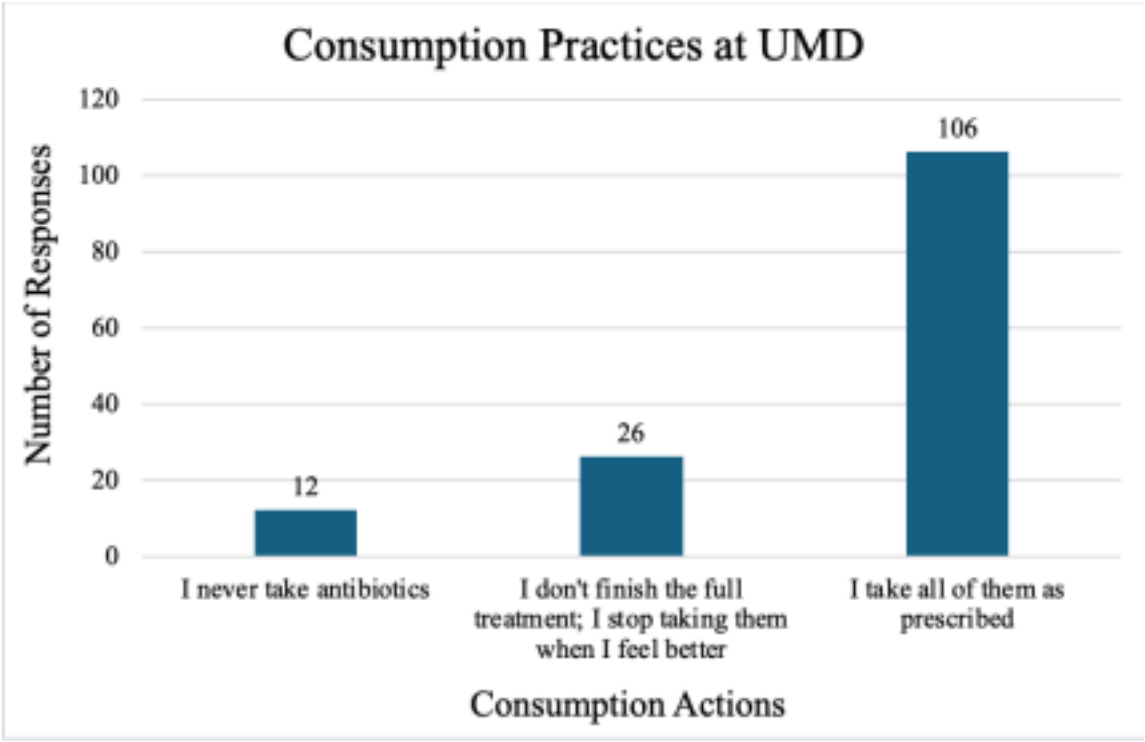

Figure 3.8 *Antibiotic consumption practices at UMCP*

The last question included in the survey was a short answer submission, asking respondents how they treat cuts or scrapes. Rather than relying on a strict one-to-one correlation between responses and treatment methods, we employed a qualitative analysis based on keyword mentions. Among the most common responses, 37 respondents in the sample size (25.69%) reported applying a band-aid, making it the most common choice. Additionally, 33 respondents (22.76%) indicated applying Neosporin to the wound, while 26 respondents (18.05%) reported cleaning the wound with soap and water as their primary course of action.

Section 3.4: Discussion

The data gathered from the public opinions survey recorded input from students enrolled at UMCP, reflecting the perspectives and practices surrounding AMR within the university

community. This student-focused survey offers valuable insights into prevailing sentiments and behaviors among this demographic, highlighting the importance of considering the educational levels and backgrounds of respondents. Students, by nature of their academic pursuits, are immersed in an educational setting where pursuit of knowledge can help shape attitudes and awareness regarding AMR. Additionally, given that many respondents are from regions within Maryland, understanding regional factors could explain how broader societal attitudes and healthcare practices influence perceptions of AMR. To be more precise, according to the Division of Research at the University of Maryland, 76.3% of the Undergraduate Student Enrollment are Maryland residents ("Facts and Figures," n.d.). Therefore, it is crucial to recognize the target audience as university students within a specific regional context, enabling findings to inform strategies not only within educational settings but also within broader community health initiatives.

Recognizing the availability of antibiotic prescriptions, or lack thereof, on college campuses is crucial for understanding campus attitudes towards AMR. 78.47% of respondents to the question “how accessible are antibiotic prescriptions to you,” indicated they have full access to antibiotic prescriptions, while only 21.53% have partial access or little to none. This variability in antibiotic access may stem from several factors, including socioeconomic status, healthcare infrastructure, and healthcare policies. Specifically looking at UMCP, health insurance coverage varies depending on the individual’s health plan. Students are required to be enrolled in the University’s insurance program or have private insurance, which can lead to varied levels of access depending on their plan benefits (“Mandatory Insurance and Waiver,” n.d.).

The frequency of responses on the public awareness of antibiotic resistance reveals a wide range of distribution among participants. The sample mean response of 6.44 regarding

understanding antibiotic resistance indicates that, on average, participants reported a moderate to proficient understanding. This suggests that the majority of respondents possess a reasonable comprehension of the topic. The variance of 5.79 reflects the degree of dispersion or spread in the responses, which indicates that there is a notable range in respondents' understanding levels about antibiotic resistance. For instance, 37.03% rated their understanding from poor to moderate. This highlights the need for an increase of antibiotic resistance awareness at UMCP, to improve overall general knowledge and understanding on the topic.

Similarly, in the assessment of participants' level of concern about the antibiotic misuse and abuse in relation to bacterial resistance, the findings demonstrate varying levels of concern. The sample mean response of 6.77 suggests that, on average, participants demonstrated moderate to high levels of concern regarding antibiotic misuse and abuse. Although the majority of respondents tend to perceive antibiotic misuse and abuse as a concerning issue, a variance of 6.18 indicates great variability in participants' levels of concern. Thus, despite the general awareness presented by the sampled population at UMCP, the data suggests that a substantial portion of participants, 39.15%, perceive antibiotic misuse and abuse as insignificant to moderately significant problems. This data could be useful for healthcare professionals to implement antibiotic resistance campaigns aimed at addressing the misuse and abuse of antibiotics at UMCP, due to the considerable number of students who might not understand the importance of this problem.

In terms of assessing the contributing factors to antibiotic resistance, participant responses shed light on consumer perception on the importance of overprescription, incorrect consumption, and overconsumption of antibiotics. The survey results imply widespread recognition of the harmful impact of these certain practices among the sample size. Regarding

incorrect consumption, only 31 respondents felt that it is a very important (rated as 10) factor for antibiotic resistance, and 60.56% of respondents displayed high understanding of incorrect consumption. Yet, this indicates that approximately 39.44% of respondents do not assume such knowledge. A lack of general awareness can indicate that people fail to complete the full course of antibiotics prescribed or use them for inappropriate purposes which will only further antibiotic resistance (Wong et al., 2021). Thus, emphasizing the importance of completing prescribed antibiotic courses and seeking medical advice before antibiotic use can help reduce the risk of spread. Additionally, practices such as overconsumption, which can be the result of self-medication, can also allow antibiotic resistance to flourish (Blaser et al., 2021, pp. 1, 11). Interestingly, at UMCP, 81.12% of the survey participants indicated moderate to proficient understanding of the important role overconsumption plays in resistance. Similar trends are displayed in regards to the importance of overprescription in antibiotic resistance. Overall, these results suggest multifaceted approaches are necessary in education and awareness programs for both consumers and providers to promote judicious antibiotic use and preserving antibiotic effectiveness.

In general, the previously mentioned results of the public opinions survey outline the awareness of antibiotic resistance within the sample size from UMCP. Generally, the trends seemed positive, indicating that the majority student sample holds considerable knowledge on the topic which can be credited to a higher level of education or SES as a college student. Yet, further research is required to understand the role of demographics within this population. Moreover, the actions of the students were also measured on the public opinions survey; over 95% of students rarely or sometimes used antibiotics. This matches the positive trend in the previous data mentioned regarding the overconsumption of antibiotics, suggesting that awareness

plays a role in consumer actions. This is exemplified by 73.61% of respondents taking all of their antibiotic treatment as prescribed, while only 18.06% stop when they feel better. The prevalence of different wound care practices were also measured in the survey. The most popular solution based on the data involved covering the wound with a bandaid, treating the wound with an antibiotic ointment, or simply using soap and water. This data was the most variable, and indicated that a substantial portion of the respondents addressed the wound to prevent further complications.

Although these findings offer valuable insights into the knowledge, attitudes, and practices surrounding antibiotic resistance within the UMCP community, the survey's respondent demographics may limit their applicability. The chosen survey distribution techniques could likely be skewed because there is the possibility that the survey was not posted or promoted equally across UMCP, presenting a potential sampling bias. Furthermore, sampling from a single university cannot yield general conclusions to anyone outside of UMCP. Therefore, it would be beneficial to expand on these initial results by expanding to more diverse communities with a variety of backgrounds with a larger sample size.

This evaluation on the current state of public awareness and knowledge at UMCP can be improved by incorporating more thorough questions to achieve a deeper level of insight into antibiotic resistance. Specifically, it would be beneficial to assess how respondents' backgrounds may factor into their awareness and decisions regarding antibiotic resistance. Alternatively, expanding on questions similar to "what do you do when you have a cut or scrape" could yield a more comprehensive analysis. Moreover, the survey can be administered to a larger sample population by exploring different survey platforms. This could be achieved by incorporating the survey into a classroom curriculum where all students, regardless of personal curiosity, are asked

to complete the survey for credit. While the survey primarily based its conclusions on a limited demographic, these results still provide an understanding of student behavior and acknowledgement of the importance of antibiotic resistance.

Chapter 4: Understanding the Effectiveness of *Aloe vera* on Bacterial Inhibition

Section 4.1: Background of Bacteria

Every layer of the skin is inhabited by bacteria (Severn & Horswill, 2023). The three main bacterial genera found on the skin are *Staphylococcus*, *Corynebacterium*, and *Propionibacterium*, which have adapted to survive in the environment of the integumentary system while maintaining a symbiotic relationship with humans (Scharschmidt & Fischbach, 2013). Of all of the *Staphylococcus* species, *S. epidermidis* are the most ubiquitous on the skin (Scharschmidt & Fischbach, 2013).

S. epidermidis is generally considered a commensal bacteria, but can also be opportunistic pathogen, capable of causing nosocomial infections as well as various skin infections or disorders (Landemaine et al., 2023). Given that *S. epidermidis* has a defense mechanism against the host's innate immune response and can serve as a source for gene transfer to *S. aureus*, it effectively increases *S. aureus*'s pathogenicity by boosting its antibiotic resistance (Otto, 2009). *S. epidermidis* traditionally was considered as the archetype of all coagulase-negative *Staphylococci*, and is more recently being studied for its contribution in clinical pathology (Severn & Horswill, 2023).

S. aureus is accountable for the great majority of skin infections, encompassing numerous conditions like cellulitis, folliculitis, impetigo, and infected ulcers, wounds, and abrasions (Krishna & Miller, 2012). About 85-95% of skin and soft tissue infections are caused by community *S. aureus* (Miller & Kaplan, 2009). Along with causing severe skin infections, *S.*

aureus is also majorly responsible for a broad spectrum of illnesses such as pneumonia, bacteremia, and various respiratory tract infections (Cheung et al., 2021). Each year within the United States 11.6 million people visit outpatient and emergency rooms due to *S. aureus* skin infection (Krishna & Miller, 2012). The immense quantities of skin infections and the rise of MRSA are cause for concern for the wellbeing of the general public (Krishna & Miller, 2012). Investigating the minimum concentration of *A. vera* needed to inhibit *S. aureus* to potentially aid in decreasing the incidence rate of skin infections and help combat antibiotic resistance. Including both a *S. epidermidis* and *S. aureus* facilitates a more comprehensive representation of the various types of *Staphylococcus* species present on the skin for this experiment.

Section 4.2: Overview of the Minimum Inhibitory Concentration Assay

The purpose of this experiment was to determine the MIC of *A. vera* and bacitracin against two common bacterial species that are found on the skin, *S. epidermidis* and *S. aureus*, to better understand *A. vera*'s antimicrobial properties by determining the lowest concentration of *A. vera* necessary to inhibit bacterial growth. *A. vera* was measured against bacitracin, a common ingredient found in many OTC topical ointment formulations given its bacteriostatic and bactericidal abilities (Nguyen et al., 2024). Thus, the addition of bacitracin was included to help compare *A. vera*'s inhibitory effects against a well-established antibiotic found in many topical ointments.

Goudarzi et al. constructed an MIC assay testing *A. vera* against multi-drug resistant strains of *P. aeruginosa* (Goudarzi et al., 2015). The researchers created a stock solution of *A. vera* with 100 g of *A. vera* gel sourced from mature leaves mixed with 1 liter of 2% DMSO. The various concentrations of *A. vera* tested were 800, 400, 200, 100, 50, and 25 µg/mL. Their

findings indicated that *A. vera*'s MIC for 89.4% of the isolates of *P. aeruginosa* strains was 200 µg/mL (Goudarzi et al., 2015). Therefore, the goal of this methodology was to model an MIC design based on the Goudarzi et al. procedure to assess the inhibitory capacity of *A. vera* against *S. aureus* and *S. epidermidis*.

Section 4.3: Methodology

To assess the potential inhibitory factors of *A. vera*, an MIC assay was designed to compare the inhibitory activity of *A. vera* and bacitracin against two pathogens: *S. aureus* and *S. epidermidis*. A glycerol *S. aureus* stock solution was transferred into a 15 mL falcon tube with 5 mL of trypticase soy broth (TSB). To isolate *S. epidermidis*, a disposable, sterile inoculation loop was used to collect three to four colonies from a Tryptic Soy Agar (TSA) plate and added into a 15 mL falcon tube with 5 mL of TSB. Both falcon tubes were incubated overnight at 37°C on a shaker at 150 rpm.

Following overnight incubation, an isolated colony of each strain was standardized by adding one colony to a culture tube containing 5 mL of autoclaved deionized water. The tube was placed into a nephelometer until the cultures were standardized to a 0.5 McFarland value. Concentrations of the inhibitory solutions were determined as 10 mg of bacitracin to 1 mL of autoclaved deionized water and 1 g *A. vera* powder to 0.5 mL of autoclaved deionized water. Both solutions were vortexed before pipetting into the plates. These concentrations were determined as the highest concentrations obtainable to pipette during preliminary experimentation.

To perform the assay, two 96-well plates were used to determine the inhibitory concentrations of *A. vera* and bacitracin. Each plate contained one of the inhibitory solutions and both species of bacteria. On both plates, a serial dilution was completed by transferring 100 µL of TSB into column 1, and 50 µL of TSB into columns 2-12. Then, 50 µL of the respective solution was added into column 1 and mixed. Then, 50 µL from column 1 was transferred into column 2 and mixed. The serial dilution was repeated in the same process, stopping at column 10. In order to test the effectiveness of *A. vera* and bacitracin, 5 µL of the bacteria (*S. epidermidis, S. aureus*) was added to each well from columns 1 through 11. Column 11 served as the positive control and received 5 µL of bacteria in the wells without a potential inhibitory solution, in order to demonstrate the bacterias' uninhibited growth in the TSB. Column 12 served as the negative control and received only 5 µL of TSB to confirm the sterility of the broth (Figure 4.1). The two 96-well plates were placed into the 37°C incubator and on a shaker at 120 rpm overnight. Bacterial growth was assessed through visual signs of cloudiness in the wells. An absence of turbidity in the wells indicated bacterial inhibition from either inhibitory solution.

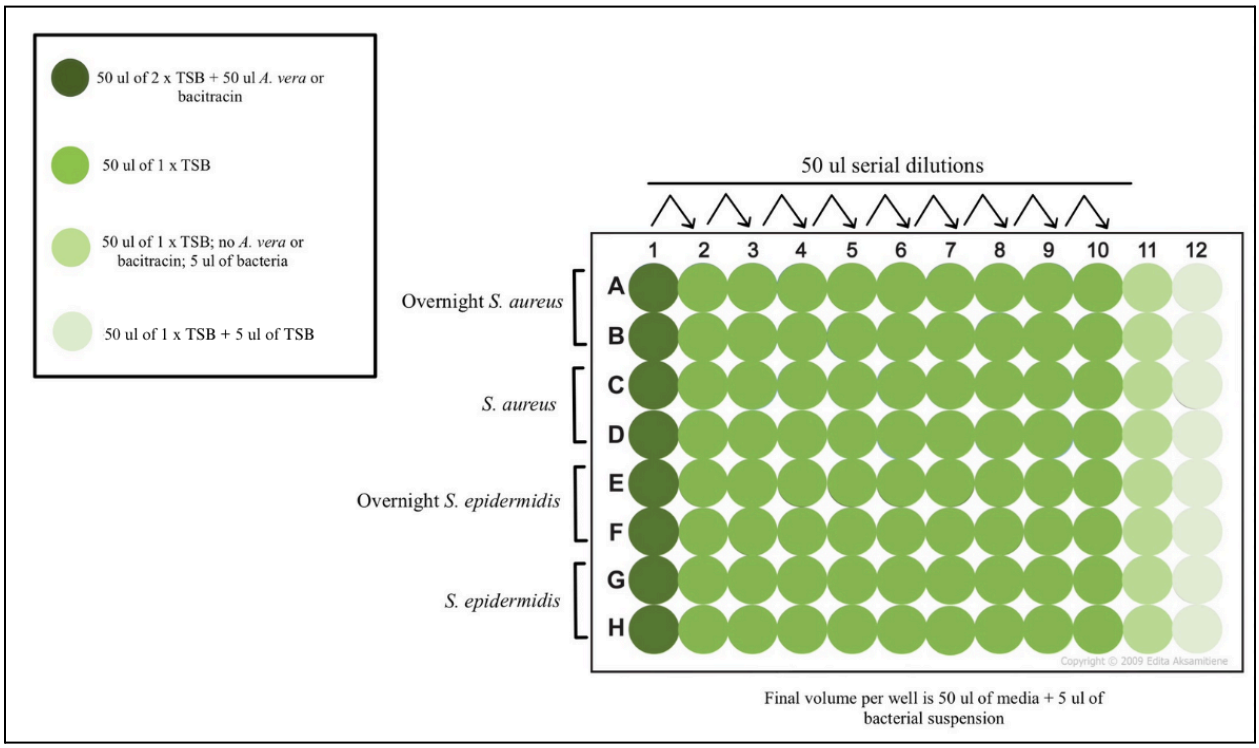

Figure 4.1: 96 Well-plate set-up for preliminary MIC assay

Section 4.4: Preliminary Results

The preliminary MIC assay proceeded over a total of three days. Two cultures of each bacterial species were tested against *A. vera* and bacitracin solutions. Rows A and B served as the experimental MIC assay for the overnight *S. aureus* culture, and rows C and D served as the experimental MIC assay for *S. aureu*s at a standardized concentration of 10^8. Consequently, rows E and F served as the MIC assay for the overnight *S. epidermidis* culture, and rows G and H were reserved for the standardized *S. epidermidis* culture at 10^8.

The bacitracin plate yielded positive results inhibiting both cultures of each bacterial species. The results determined were based on if the wells expressed turbidity on day three by visual confirmation. At a 1:2 ratio of bacitracin to deionized water, the bacitracin solution inhibited almost all of the bacterial colonies. Figure 4.2 and 4.3 demonstrate turbidity expressed

in three of the overnight cultures in wells A10, B10, and E10 while well F10 did not express signs of turbidity.

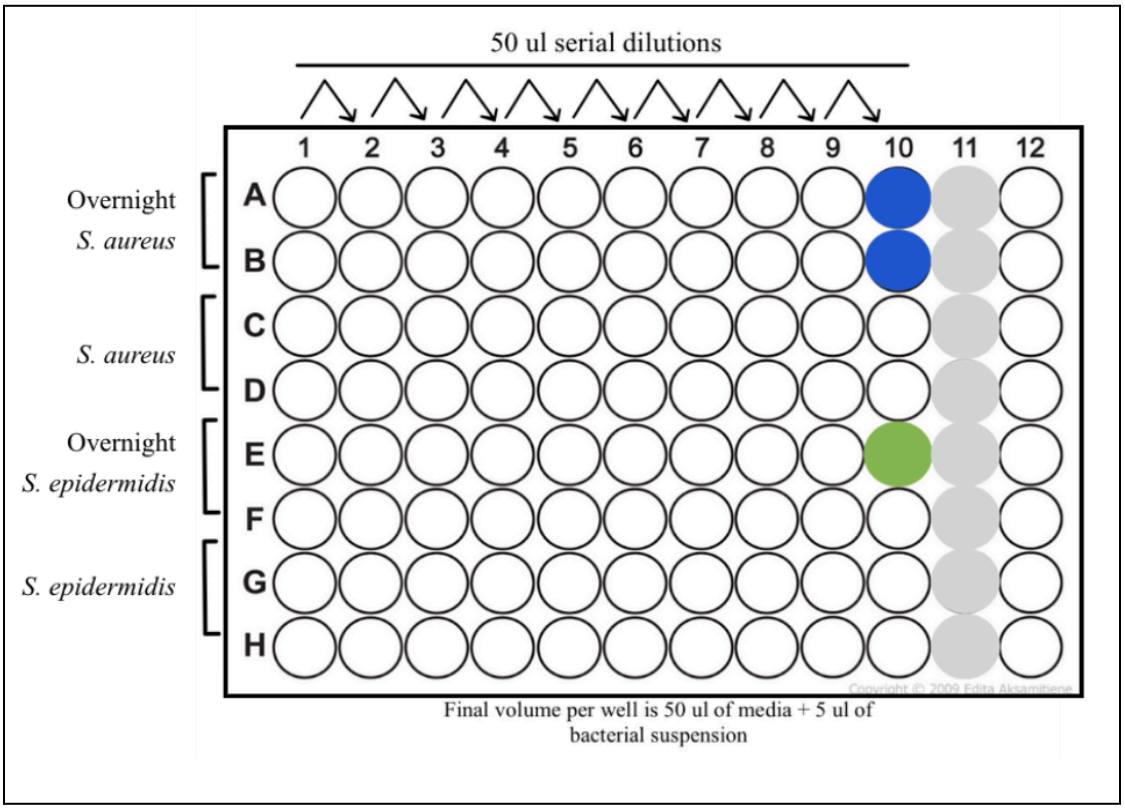

Figure 4.2 *Bacitracin MIC Assay Against Staphylococcus aureus and Staphylococcus*

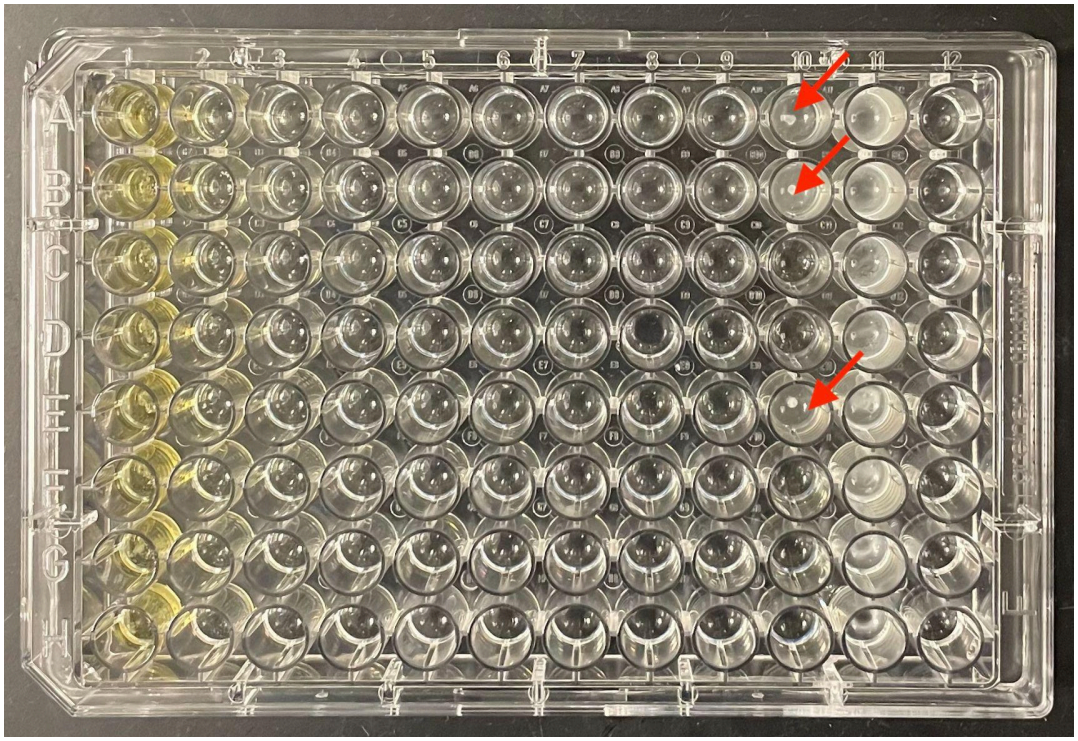

Figure 4.3: *Day 3 Photograph of Bacitracin MIC Assay Against Staphylococcus aureus and Staphylococcus epidermidis*

The *A. vera* plate did not show signs of inhibition of the two cultures of either bacterial species. At a 1:2 ratio of *A. vera* to deionized water, the *A. vera* solution did not indicate inhibition of bacteria. Figure 4.4 and 4.5 highlight the results on day three of the experiment with turbidity in all of the wells for each bacterial sample except wells E6 and H2.

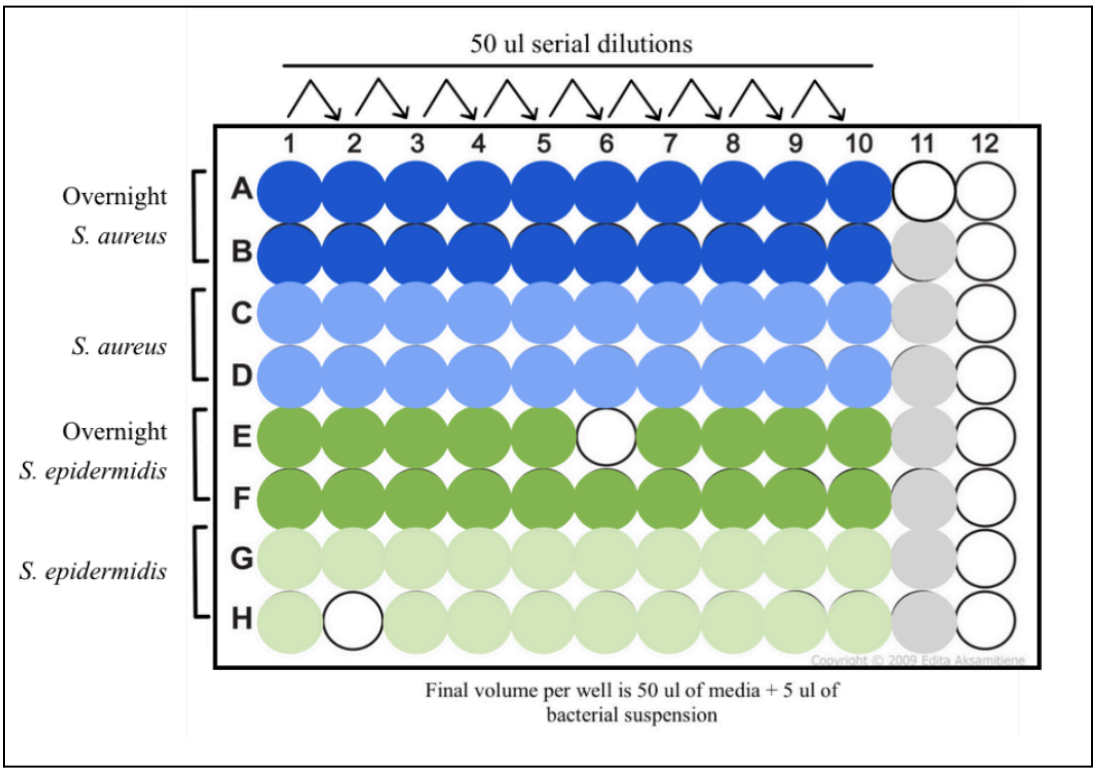

Figure 4.4 *Aloe Vera MIC Assay Against Staphylococcus aureus and Staphylococcus epidermidis*

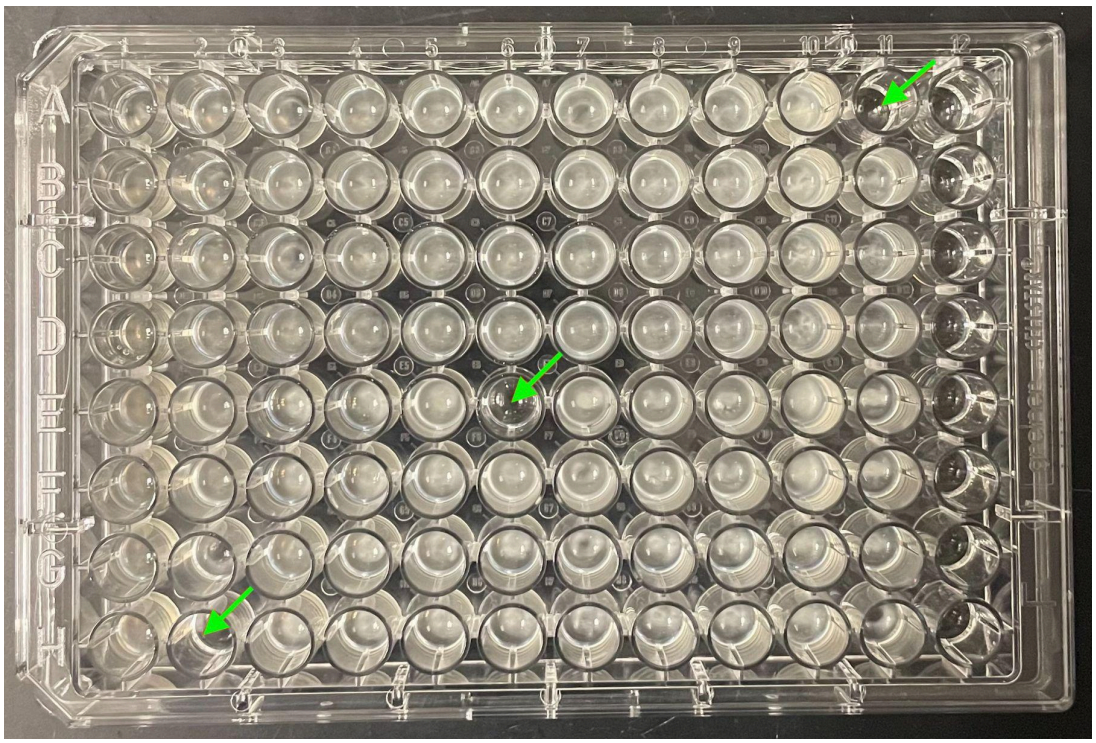

Figure 4.5: *Day 3 Photograph of Aloe Vera MIC Assay Against Staphylococcus aureus and Staphylococcus epidermidis*

Section 4.5: Discussion

This experiment did not offer conclusive results regarding *A. vera*'s bacteriostatic abilities against *S. aureus* and *S. epidermidis*. Due to the absence of a plate reader necessary to quantitatively assess the bacterial colonies, results were qualitatively analyzed through visual measurements of turbidity. From the preliminary data collected, an MIC value was unable to be determined since the concentration of bacitracin was too potent for the bacterial species. Since the overnight cultures in A10, B10, and E10 had signs of turbidity but an unknown number of colonies, it is unfeasible to determine the MIC value of bacitracin. Also, as seen in Figure 4.2, the standardized cultures were inhibited across the plate, also emphasizing that an MIC value cannot be determined, even with a known value of bacterial colonies. Additional experimentation is essential to identify the MIC value of bacitracin against *S. aureus* and *S. epidermidis*. Additionally, it can be inferred that the MIC value of *A. vera* is higher than the MIC value of bacitracin, since the *A. vera* was unable to inhibit both bacterial species. Further trials are necessary to ascertain whether *A. vera* can effectively inhibit the growth of both *S. aureus* and *S. epidermidis*. Relying solely on this singular dataset makes it challenging to conclusively determine the antimicrobial capabilities of *A. vera* against these two pathogens.

There were several administrative challenges when preparing for the bacterial inhibition experiment. Firstly, it was difficult and time-consuming to find a laboratory that allowed for experimentation with *S. aureus* since it is a biosafety level 2 pathogen. In addition, there were challenges acquiring the bacterial specimens because of regulation guidelines that necessitated Federal Drug Administration (FDA) approval in the form of a United States Department of

Agriculture (USDA) permit that the bacterial experimentation team did not have. The bureaucratic procedures associated with obtaining such a permit made it virtually impossible to acquire bacteria specimens without external assistance. Thus, both of these obstacles effectively hindered the start of the experiment and should be addressed for future research.

Furthermore, there was an obstacle with the experimental design. When attempting to raise the concentration of the *A. vera* solution, by increasing the ratio of the *A. vera* powder to deionized water, it became too viscous to pipette into the 96-well plates. This impediment resulted in an inability to test higher concentrations of the *A. vera* against the target bacterial species.

Moreover, the differential results could be related to the *A. vera* powder used. Arbab et al. determined the zones of inhibition of various bacteria, including *S. aureus,* by performing a disk diffusion technique with *A. vera* leaf and root extracts (Arbab et al., 2021). However, the *A. vera* powder utilized in this experiment did not specify what part of the *A. vera* plant it derived from nor how it was collected, potentially affecting *A. vera's* antimicrobial effects and possibly leading to results that conflicted with the literature review. Additionally, it was not compatible with certain solvents like ethanol which perhaps when added, could have aided in yielding positive results that correspond with previous literature.

To address these limitations for future experimentations, it is imperative to delve deeper into the specifications of the *A. vera* powder and gels used and change the source and application of *A. vera* and if necessary to align with literature. As mentioned previously, Arbab et al. emphasized the antimicrobial potency of *A. vera* extracts sourced from the leaf and root, especially when created with the use of ethanol (Arbab et al, 2021). Using an *A. vera* powder

with ethanol may enable more flexibility in adjusting concentrations, and hopefully yield positive results.

Chapter 5: Understanding the Role of *Aloe vera* on Dermal Fibroblast Proliferation

Section 5.1: Overview of the Dermal Fibroblast Proliferation Preliminary Assay

Previous studies have shown that *A. vera* can induce dermal fibroblast (DF) proliferation both *in vitro* and *in vivo*. Shafaie et al. indicated that raw *A. vera* gel can increase the migration of DF, which aligns with cellular actions during the proliferative phase of wound healing (Shafaie et al., 2020). Additionally, *A. vera* extract has been found to accelerate wound healing and increase DF count, hence promoting re-epithelialization after surgically-inducing lacerations on rats (Girsang et al., 2020). For the most part, there is substantial evidence that associates *A. vera* with DF proliferation. The following methodology was designed to determine whether there is an optimal concentration of *A. vera* for increasing skin cell proliferation as current literature outlines various amounts in different states. For this preliminary assay, DF growth was measured when exposed to *A. vera*, bacitracin, and methylene blue (MB) to establish *A. vera*'s baseline proliferative abilities.

MB was used as an additional positive control due to its substantial impact on fibroblast proliferation. A 2007 study tested 10 nM, 100 nM, and 1000 nM MB on lung fibroblasts and found that MB was associated with higher oxygen consumption, elevated levels of mitochondrial cytochrome C oxidase, increased heme synthesis, and overall improved cellular protection among fibroblasts treated with the higher concentrations of MB (Atamna et al., 2007). Xiong et al. built on these results by testing the effects of 100 nM MB on DF lines derived from individuals over 80 and under 30 years old, finding that MB was significantly more effective in promoting fibroblast proliferation and delaying senescence compared to more commonly used

antioxidants (Xiong et al., 2017). Although MB and *A. vera* are suggested to act through different mechanisms of action in promoting DF growth, comparing the impacts of both will allow for a comprehensive understanding of their respective contributions to wound healing. Bacitracin and growth media were used as negative controls to show that while cyclic antibiotics, like bacitracin found in topical ointments, exhibit bacteriostatic and bactericidal properties, these compounds do not necessarily promote cell proliferation (Nguyen, 2022).

Prior research indicates that an *A. vera* extract in a growth media percentage of 1.2- 2.5% yielded an increase in diabetic DF proliferation while other research indicates a 50- 96.4% gel concentrate (Jamil et al., 2018; Abdullah et al., 2003). Since the *A. vera* for the experiment was in powder form, a 2.5% w/v concentration of it in culture media was chosen as a baseline value for the preliminary assay.

Section 5.2: Methodology

To assess if *A. vera* aids in cell proliferation, a preliminary assay was designed to measure *A. vera*'s proliferative capacity on DF (passage 17) isolated from a 50 to 60-year-old female. After thawing, cells were plated on a 100 mL dish in glucose-rich DMEM and 10% FBS growth medium, grown in an incubator set at 37℃ in 5% CO_2, and monitored until reaching approximately 60% confluence. The media was changed approximately every 48 hours. At confluence, four 12-well plates were assembled to monitor the treatment conditions (*A. vera,* bacitracin, MB, and growth media) over the course of four days (Figure 5.1). Roughly 25,000 cells were seeded within each well, with each condition produced in triplicate.

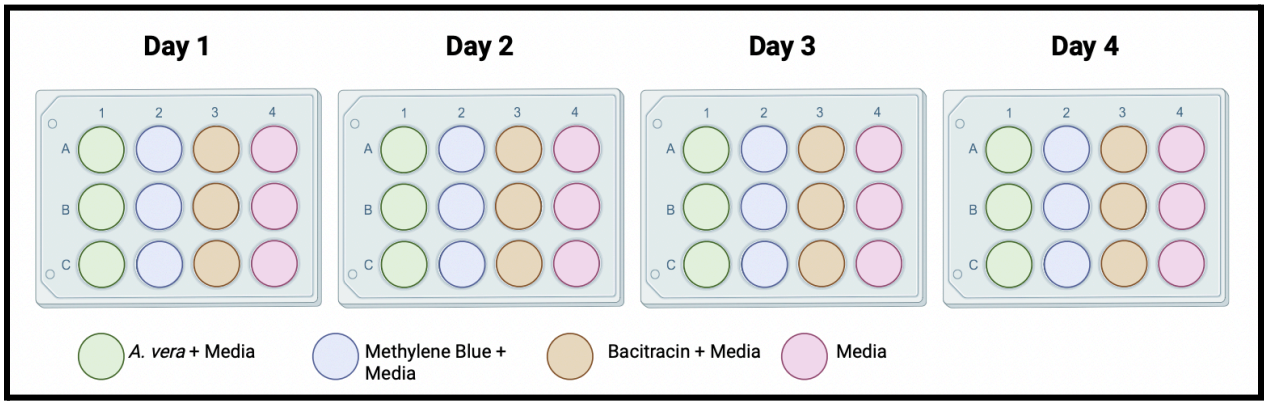

Figure 5.1: *12-well plate Schema for Four Day Experiment for DF proliferation assay*

A. vera stock solution was prepared at 500 mg/mL by hydrating 0.5 grams of anhydrous *A. vera* powder with diH_2O. The bacitracin condition was created at 200 mg/mL stock solution through the dissolution of 2 grams of anhydrous bacitracin into 10 mL of diH_2O. To produce a working bacitracin solution for use within the seeding protocol, 10 µL of stock bacitracin was diluted with 10 mL of DMEM media. Following preparation of stock treatments which were created using an anhydrous powder, solutions were filtered utilizing a sterile syringe. The MB condition was created by performing a 1:1000 dilution of a 100 µM MB aliquot with DMEM.

To seed each well within the 12-well plates, old media for the P.17 DF sample - which was grown on a 100 mL dish- was removed via vacuum, after which the sample was washed with PBS. The sample was then trypsinized utilizing 1 mL of trypsin for approximately four minutes, where cells were then resuspended through the addition of 6 mL DMEM to halt the trypsin reaction. Cells were then spun down via centrifuge to ensure the complete removal of trypsin. Supernatant was then removed and the pellet was resuspended in 2 mL of DMEM. Cell count was approximated at $1.2 \cdot 10^6$ cells within the 2 mL of resuspended DF solution. The 2 mL cell suspension was then diluted to 24 mL through the addition of 22 mL DMEM media.

Individual wells were seeded with 500 µL of resuspended cell solution, equating to approximately 25,000 cells per well.

To produce the *A. vera*, bacitracin, and MB conditions within each column, each well was treated with 500 µL of working stock solution to produce respective concentrations of 2.5% w/v *A. vera*, 100 nM bacitracin, and 100 nM MB. Four replicate plates were produced in order to record measurements over the course of four days, as cell counting would implicitly entail contamination of the sample (Figure 5.1).

Section 5.3: Preliminary Results

The results of the DF preliminary assay indicate a rejection of all three possible null hypotheses of a two-way ANOVA statistical test. The null hypotheses are as follows:

H_0: Treatments will have no significant effect on fibroblast cell count.
H_0: Number of days will have no significant effect on fibroblast cell count.
H_0: Treatment and number of days will have no significant effect on fibroblast cell count.

Based on the analysis, and considering the interaction between treatment and day, the F distribution (9, 128) = 3.335626 indicates a P-value of 0.001075 < P(0.05). In regards to the treatment factor, the F distribution (3, 128) = 2.675387 which correlates with a P-value of 0.011388 < P(0.05). Finally, for the day factor, the F distribution (3, 128) = 2.675387 which corresponds to a P-value of 4.31E-11 < P(0.05) (Table 5.1).

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Treatment	2.13E+10	3	7.09E+09	3.836413	0.011388	2.675387
Day	1.16E+11	3	3.87E+10	20.91732	4.31E-11	2.675387
Interaction	5.55E+10	9	6.17E+09	3.335626	0.001075	1.953763
Within	2.37E+11	128	1.85E+09			
Total	4.29E+11	143				

Table 5.1: *Two-way ANOVA analysis with treatment groups and day as different variables*

There was notable variability among the cell count values for the DF preliminary assay. On day one, the control group displayed a mean value of 6.73E+4 ± 14120.02 cells, *A. vera* 8.62E+4 ± 67505.26 cells, bacitracin 8.16E+4 ± 24723.12 cells, and MB 1.06E+5 ± 69567.04 cells. On day two, the control group displayed a mean value of 9.09E+4 ± 21652.89 cells, *A. vera* 7.30E+4 ± 27360.97 cells, bacitracin 3.39E+4 ± 42923.30 cells, and MB 4.14E+4 ± 28127.40 cells. On day three, the control group displayed a mean value of 4.33E+4 ± 16094.78 cells, *A. vera* 7.30E+4 ± 32698.30 cells, bacitracin 3.39E+4 ± 8997.98 cells, and MB 4.14E+5 ± 17946.03 cells. On day four, the control group displayed a mean value of 1.17E+5 ± 58092.66 cells, *A. vera* 1.91E+5 ± 75388.62 cells, bacitracin 1.04E+5 ± 61281.48 cells, and MB 8.75E+4 ± 31291.17 cells. The data can be visualized for trends (Figure 5.2).

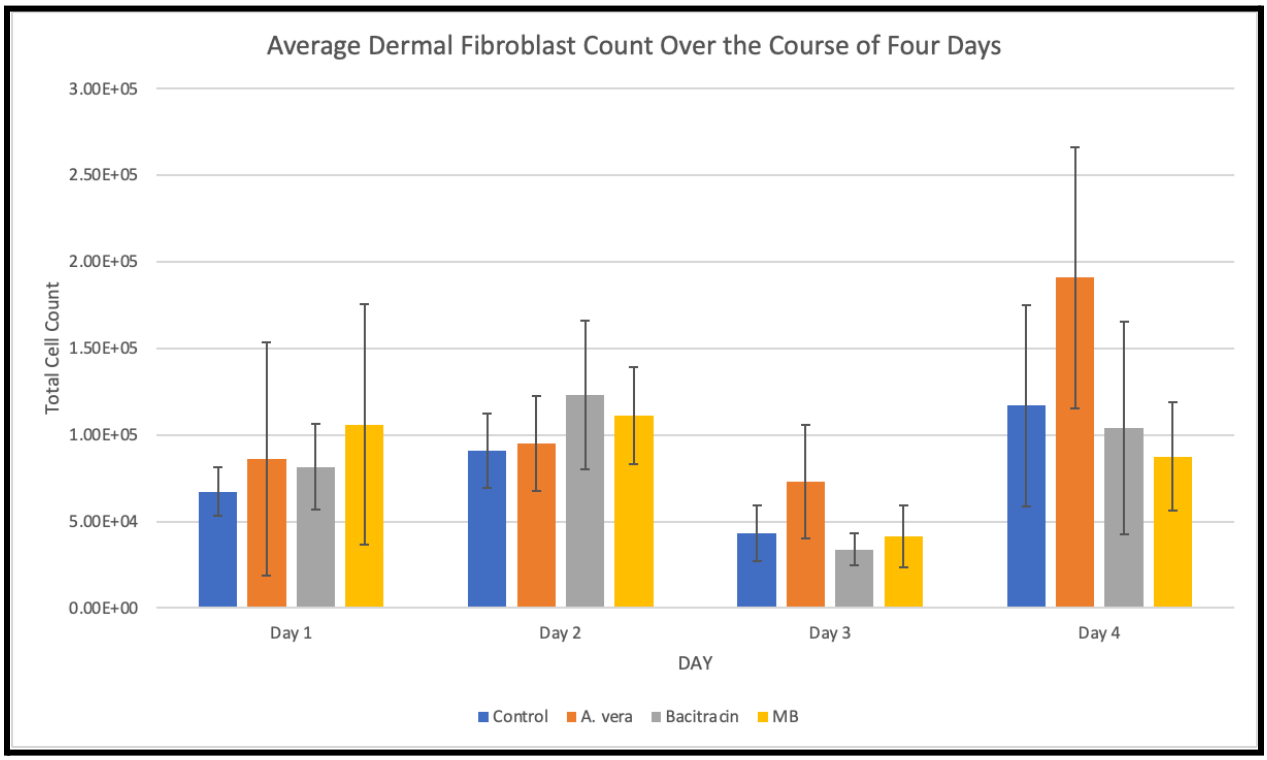

Figure 5.2: *Mean cell count of DF in four treatment groups over the course of four days*

Between days one and four, the *A. vera* supplemented cell population grew by 1.05E+5 ± 101194.9 cells: the largest growth observed in each condition. In the same timeframe, growth media and bacitracin treatment cell counts increased by 4.97E+4 ± 59784.05 and 2.24E+4 ± 66080.65 cells, respectively, while MB experienced a 1.85E+4 ± 76280.47 cell decrease.

Section 5.4: Discussion

The preliminary DF proliferation assay does not provide conclusive results regarding *A. vera*'s capacity for increasing cell proliferation. The two-way ANOVA analysis indicates the rejection of all three null hypotheses, indicating that there is a statistically significant change in cell count, but further analysis is necessary to determine which groups are statistically different from one another, especially considering the variability and large deviations between treatment conditions and days (Figure 5.2).

In literature, bacitracin is not associated with wound-healing abilities; its primary function is to inhibit bacterial cell wall synthesis (Nguyen, 2022). Since no microbes were used during the DF preliminary assay, it was assumed that the cells in the bacitracin and growth media control groups would proliferate at the same rate. The data suggested aligns with this assumption: there is no statistically significant difference between the cell count of the bacitracin between each day, aside from day 3 which was reduced for all conditions. However, cells grown in MB and *A. vera*-supplemented media did not align with literature-based expectations. While *A. vera*-supplemented populations increased between day one and day four, the change was not statistically significant (Figure 5.2). Strikingly, despite MB's established positive influence on DF proliferative properties, MB-supplemented cell populations experienced a count reduction between day 2 and day 3. This dissonance between observations and literature exemplifies the need for further repetition and modification to experiment protocols.

Experimental error could be a principal contributor to the lack of certainty presented by the DF preliminary assay. During cell culture days two and three, the DF in the *A. vera* treatment group were particularly difficult to detach from the surface of the plate after trypsinizing the wells compared to other treatment groups. This effect may have been caused by increased cell adhesion induced by upregulated α_1 and β_1 integrin synthesis by *A. vera*, however, this does not explain the ease of trypsinization experienced on day four (Shafaie et al., 2020). Achieving majority detachment required incubating the sample in Trypsin beyond the recommended incubation period, possibly damaging the cells while failing to achieve full sample collection. On the same culture days, it was observed that the plate assigned for counting on day 3 had consistent cell separation across each experimental condition. This could have been induced by

mechanical damage inflicted when moving plates, potentially causing the uniform decrease in total cell counts for that day.

Additionally, the data is based on the total cell count on the Luna-FI dual fluorescent cell counter machine. Although the live cell count and viability was measured, it was not considered in the data analysis due to the absence of Trypan Blue. During procedure development, it was noted that Trypan Blue appeared to lyse the DF cells when observed on the Luna-FI cell counter, leading to its exclusion in sample preparation. This effect may have been caused by degradation of the Trypan Blue due to reagent age. Excluding Trypan Blue led to reliance on total DF counts in data analysis and decreased data quality, possibly contributing to the significant variability observed in Figure 5.2. For future experiments, it will be necessary to utilize fresh Trypan Blue, to obtain accurate readings of live cells and inferences about each conditions' impact on viability.

The manufacturing process to produce *A. vera* powder may have contributed to some variation in these results. For the preliminary assay, *A. vera* powder was used due to its accessibility and ease of use. The powder was manufactured through pasteurizing *A. vera* gel and concentrated with low temperature evaporation and freeze-drying techniques (Spectrum Chemical, n.d). The *A. vera* concentrations chosen for the experiments were based on previously conducted studies, most of which used *A. vera* gel for assay-based experiments rather than powder. Variations in processing and handling among brands of *A. vera* powder may cause deviations in active compound composition compared to fresh gel. One study by Bozzi et al. assembled ^{1}H NMR profiles for nine *A. vera* gel powder concentrates produced by different manufacturers, measuring their acemannan, malic, acid, and glucose content (Bozzi et al., 2007). Their results established significant differences in quality between the powders: only three

products contained satisfactory amounts of acemannan (at least 10% w/w), with the remaining samples having only 1-5%. Moreover, four contained over 10% w/w lactic acid, a sign of bacterial fermentation (Bozzi et al., 2007). Although Bozzi et al. did not disclose manufacturer identities, their work raises concerns about the reliability of *A. vera* powders available for research purposes. Choosing to use fresh *A. vera* gel may mitigate some of this risk, but there is limited research comparing how different concentrations of fresh gel and whole leaf powder extracts influence bacterial inhibition and fibroblast growth.

Chapter 6: Conclusion

Section 6.1: Conclusion

The purpose of this project was to examine *A. vera* as a potential research focus in the larger context of AMR R&D and stewardship. The goals encompassed four key objectives: compiling a comprehensive review of AMR and its connection to research on *A. vera*, highlighting antibiotic resistance knowledge and practices at an accessible community, ascertaining *A. vera's* inhibitory properties on specific bacterial species, and assessing if *A. vera* contributes to skin cell proliferation to serve a dual role in wound healing.

The literature review on AMR highlighted the pressing need for AMR education and stewardship for consumers and stakeholders, respectively. Minimizing the impact of AMR by upholding the integrity of antimicrobial medicines requires a combination of public outreach and drug development efforts. To identify target areas for educational intervention, an assessment was conducted to evaluate the current state of antibiotic resistance awareness and practices at UMCP. The results of this survey suggest that students at UMCP have moderate to high awareness and understanding of antibiotic resistance and its consumer-related causes. Further assessment is necessary to account for variables within the student demographics, such as SES, that may lead to differences in AMR awareness. Adopting explicit metrics for the interpretation of the question, increasing the sample size, and enhancing the survey demographics would provide a more comprehensive data set.

The MIC and dermal fibroblast proliferation assays aimed to provide insight into how *A. vera* can serve a dual role as an antibacterial and wound-healing agent. Extensive literature on the bacterial inhibitory properties of *A. vera* and its biologically active compounds indicated its potential as an antibacterial agent. Yet, the MIC assay designed to study the influence of *A. vera*'s potential inhibitory properties on *S. epidermidis* and *S. aureus* yielded inconclusive results due to experimental limitations. To improve on the experimental techniques, it would be beneficial to explore different states of *A. vera* by combining it with other reagents. Moreover, future experiments can include a plate reader to quantify the amount of bacterial cells instead of relying on visual signals and multiple trials to ensure validity of results. In general, further investigation is necessary to explore the role of *A. vera* in bacterial inhibition to control AMR.

Similarly, the preliminary DF assay did not produce results that aligned with literature expectations. DF cultured in 2.5% w/v *A. vera*-supplemented media alongside DF cultured in 100 nM MB and 100 nM bacitracin did not show conclusive directional results in cell count over the culture period. Direct examination of *A. vera*'s active compounds, such as acemannan, have been shown to increase cell proliferation, suggesting a need for assay protocol modification. Exploring different preparations of *A. vera*, particularly fresh *A. vera* gel, and performing multiple replicate experiments may increase the reliability and consistency of assay results. Future researchers could explore using a more diverse range of cell attachment methods, varying incubation conditions, and cell culture media formulations, which will provide a more exhaustive and insightful experiment to understand the impact of *A. vera* on wound-healing.

Overall, the components discussed outline the severity of AMR and the fundamental responses needed to address the global health problem. This *silent pandemic* impacts millions a year, and is only expected to worsen (Tang et al., 2023). Therefore, it is vital that researchers

focus their efforts to explore novel solutions while promoting antimicrobial stewardship. Sourcing the abundant medicinal properties of plants, like *A. vera*, is a promising path. Although the results of this research are preliminary and fundamental, it provides a baseline and framework for future research.

Section 6.2: Note to Future Undergraduate Students

The Gemstone Honors Program at the University of Maryland is committed to creating an interdisciplinary research community for undergraduate students. As future researchers, this exposure to the different aspects of the research process can be daunting and confusing. Team Aloesporin recommends the following pieces of advice to any undergraduate student interested in pursuing a long-term project:

1. Establish effective communication and collaboration techniques to maximize research output when working with others.
2. Adjust and adapt to any roadblocks faced during the administrative and experimental processes; science is a trial and error field which requires a lot of creativity and determination.
3. Allow yourself to grow into the role of a research scientist without expectations that can restrict your ability to learn.

Overall, Team Aloesporin encourages future students to seize the opportunity to contribute to the ongoing fight against antimicrobial resistance, leveraging their unique perspectives and skills to drive innovation and discovery in this critical field and finding a way to improve on the current state of research.

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