Bindura University of Science Education

The Effects of Ceftriaxone and Ciprofloxacin Antibiotics Concentration levels on *Staphylococcus aureus* Growth and Resistance

By

NIGEL. S. LIBELATO

B213198B

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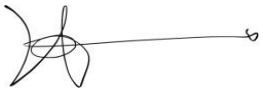
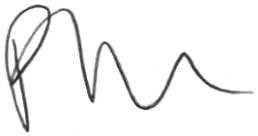
Signature of student :  Date : 14 June 2025

Signature of Supervisor :  Date : 20/08/25

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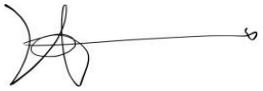
Signature:  Date: 14 June 2025

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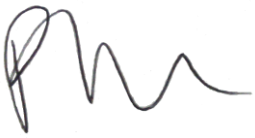
Signature:  Date: 20/08/25

Dedication

I dedicate this project to my beloved family whose encouragement and support have been the foundation of my perseverance and success.

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List of abbreviations

MRSA: Methicillin- Resistant *Staphylococcus aureus*

MSSA: Methicillin-Susceptible *Staphylococcus aureus*

MIC: Minimum Inhibitory Concentration

CFU: Colony Forming Units

DNA: Deoxyribonucleic Acid

RNA: Ribonucleic Acid

CLSI: Clinical and Laboratory Standards Institute

ANOVA: Analysis of Variance

PPE: Personal Protective Equipment

List of tables

List of figures

ABSTRACT

The study investigated the effects of varying concentration levels of ceftriaxone and ciprofloxacin antibiotics on the growth and resistance development of *Staphylococcus aureus* isolates. The experiment was conducted using Methicillin Susceptible *Staphylococcus aureus* (MSSA) and as well as the Methicillin Resistant *Staphylococcus aureus* (MRSA) strains obtained from Bindura Provincial Hospital and Shashi Hospital. Antimicrobial activities were assessed by measuring the diameter of inhibition zones on Mueller Hinton agar using antibiotic-impregnated discs at low and high concentrations. Bacterial samples were then re-exposed to standard therapeutic doses to evaluate changes in susceptibility. Quantitative results showed that both antibiotics effectively inhibited *S. aureus* growth, with combination treatments exhibiting the greatest zones of inhibition. Higher concentrations significantly enhanced antibacterial efficacy compared to lower concentrations. Upon re-exposure, bacteria previously subjected to low concentrations exhibited reduced inhibition zones, indicating resistance development. Statistical analysis using one-way ANOVA and Tukey's post hoc test confirmed significant differences among treatment groups ($p < 0.001$). The findings suggest that sub-lethal antibiotic exposure contributes to the emergence of resistance in *S. aureus*, while combination therapy and appropriate dosing improve treatment effectiveness. The study highlights the clinical importance of antibiotic stewardship in minimizing resistance and supports further research into combination therapies and resistance mechanisms.

CONTENTS

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

The Gram positive bacteria *Staphylococcus aureus* is a major contributor to infections that are obtained in the community and those that are linked to healthcare institutions. *S. aureus* infections can result in a variety of symptoms, starting with minor infections of the skin and soft tissues to serious conditions like pneumonia and bloodstream infections. *S. aureus* can cause a wide range of disorders due to its virulence components, which include adhesions, digestive enzymes, and toxins Jones (2019). Because it can develop resistance to a variety of antibiotics, including fluoroquinolones, beta-lactams, and other kinds of antibiotics, *Staphylococcus aureus* is one of the numerous forms of Staphylococcus that is a concern.

Two common medicines used to treat infections brought on by gram-positive bacteria like *S. aureus* are ciprofloxacin and ceftriaxone. Ciprofloxacin, a fluoroquinolone, inhibits the replication of bacterial DNA, whereas ceftriaxone, a third-generation cephalosporin, inhibits the development of bacterial cell walls causing them to burst and die. Both antibiotics have been used widely because they are efficient in treating a variety of bacterial diseases. The development and spread of antibiotic resistance in *S. aureus*, however, poses a significant threat to the efficacy of treatment.

Concern has been raised in recent years about the growing number of antibiotic-resistant *S. aureus* strains, such as methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA). These resistant strains are increasing mortality, disease, and healthcare costs. Understanding how antibiotics affect *S. aureus* growth and resistance mechanisms is essential for developing strategies to treat antibiotic-resistant infections and for making decisions about antibiotic therapy.

The effects of ceftriaxone and ciprofloxacin on *S. aureus* growth and resistance have been investigated in a number of research. In this respect, Smith (2018) explored into the synergistic effects on multidrug-resistant *S. aureus* strains with ceftriaxone and ciprofloxacin combination therapy. According to their research, using both antibiotics at once may be able to suppress resistance processes and improve the killing of bacterial infections.

On the other hand, after extended antibiotic exposure, ciprofloxacin-resistant *S. aureus* isolates were shown to arise, according to a study conducted by Jones (2019). This researcher found changes in the genes for topoisomerase IV and bacterial DNA gyrase, which may have contributed to the decreased susceptibility to ciprofloxacin (Jones, 2019).

Furthermore, research by Brown, (2020) and White (2021) shown how efflux pumps mediate *S. aureus* resistance to ciprofloxacin and ceftriaxone. Antibiotics are actively pumped out of bacterial cells by these membrane transporters, which lowers intracellular drug concentrations and decreases therapeutic efficiency.

Further investigation has been done by researchers like Garcia, Martinez, & Perez (2018) to determine the genetic basis of ceftriaxone and ciprofloxacin resistance in *S. aureus*. Their research pinpointed certain resistance genes, mutations, and patterns of gene expression linked to antibiotic resistance, offering valuable information about putative treatment targets.

Also, the impact of antibiotic exposure on *S. aureus* biofilm formation and virulence has been studied by Patel, Brown, & Gray, (2020). These researchers demonstrated that sub-inhibitory concentrations of ceftriaxone and ciprofloxacin could enhance biofilm formation and promote bacterial persistence, leading to treatment failure in chronic infections.

The literature underscores the complex interplay between ceftriaxone, ciprofloxacin, *S. aureus* growth, and antibiotic resistance. This research project aims to further elucidate the effects of these antibiotics on *S. aureus* isolates, exploring their impact on bacterial growth dynamics, resistance mechanisms, and virulence factors.

1.2 STATEMENT OF PROBLEM

The rise of antibiotic resistant *Staphylococcus aureus* forms, particularly MRSA, poses a major threat to global public health. There are antibiotics like ceftriaxone and ciprofloxacin that can be used to treat *S. aureus* infections, but drug resistance is becoming a bigger issue. Treatment of infections brought on by *Staphylococcus aureus* infections is severely hampered by the bacteria' increased drug resistance. There aren't many thorough studies in the literature right now that explain how different antibiotic concentrations affect *S. aureus* growth dynamics and resistance mechanisms. Existing research predominantly focuses on standard clinical doses of antibiotics, often neglecting the effects of varying concentrations on bacterial growth and resistance. Resistance mechanism insights on most studies do not delve deeply into the mechanisms by which *S. aureus* develops resistance to ceftriaxone and ciprofloxacin at different concentration levels. This leaves a critical gap in understanding the full spectrum of bacterial responses to these antibiotics. There is a lack of comparative studies examining the impacts of ceftriaxone and ciprofloxacin concentrations on the same bacterial strains, which is essential for optimizing treatment protocols. The urgent need to address the growing issues posed by antibiotic-resistant strains of *Staphylococcus aureus* provides rationale for examining the effects of ceftriaxone and ciprofloxacin drugs on *S. aureus* development and resistance. This knowledge is crucial for developing strategies to counteract

resistance. Without this vital information, doctors are frequently forced to base their treatment choices on imprecise or generalized data, which can result in less-than-ideal results and the continued emergence of resistance. In order to improve our knowledge of antibiotic resistance in *S. aureus* and offer insights that could guide the development of more potent treatment strategies for the treatment of infections caused by resistant *S. aureus*, it is essential to explore the mechanisms of resistance and the impact of various antibiotic concentrations on bacterial growth and resistance profiles.

1.3 AIM

The purpose of this study is to investigate the effects of ceftriaxone and ciprofloxacin concentration levels on *Staphylococcus aureus* growth and development as well as the emergence of antibiotic resistance. It seeks to clarify the mechanisms underlying the bacteria's response to ceftriaxone and ciprofloxacin treatment. More specifically, the study intends to identify any changes in resistance profiles that may occur after antibiotic exposure and ascertain how these antibiotics affect the growth dynamics of *S. aureus* strains.

1.4 OBJECTIVES

1. To determine the inhibition zone of *Staphylococcus aureus* growth under different ceftriaxone and ciprofloxacin concentrations.
2. To assess whether there is a significant difference in the inhibition zones among bacteria exposed to different antibiotic concentrations.
3. To evaluate the inhibition zones of *Staphylococcus aureus* after pre-exposure to different concentrations of ceftriaxone and ciprofloxacin, assessing any potential changes in bacterial resistance.
4. To determine whether *Staphylococcus aureus* inhibition zones differ significantly from one another after pre-exposure to various antibiotic concentrations, focusing on any potential changes in bacterial resistance.

1.5 RESEARCH QUESTIONS?

1. What are the inhibition zones of *Staphylococcus aureus* when exposed to different concentrations of ceftriaxone and ciprofloxacin?
2. Is there a statistically significant difference in the inhibition zones of *Staphylococcus aureus* exposed to varying concentrations of ceftriaxone and ciprofloxacin?

3. How does pre-exposure to different concentrations of ceftriaxone and ciprofloxacin affect the inhibition zones of *Staphylococcus aureus*?
4. Does pre-exposure to varying concentrations of ceftriaxone and ciprofloxacin lead to significant changes in the resistance patterns of *Staphylococcus aureus*, as reflected by inhibition zone measurements

1.6 HYPOTHESIS

H_0: No significant differences exist in inhibition zones among *Staphylococcus aureus* cultures pre-exposed to different antibiotic levels.

H_1: Significant differences exist in inhibition zones among *Staphylococcus aureus* cultures exposed to varying concentrations, including those pre-exposed to different antibiotic levels.

1.7 SIGNIFICANCE OF STUDY

The importance of this work stems from its ability to improve our knowledge of the intricate connection between the use of ceftriaxone and ciprofloxacin, the growth of *Staphylococcus aureus*, and the emergence of resistance. By investigating the effects of these 2 antibiotics on *S. aureus* growth and resistance, this research has the potential to provide valuable information about the factors that underlie the development of the antibiotic's resistance in *S. aureus* populations, leading to more targeted and effective treatment strategies. It also helps to identify potential risk factors and patterns of resistance emergence, informing the antibiotics prescribing practices and minimizing the spread of multidrug-resistant bacteria. In order to effectively battle resistant bacterial strains, the study aids in the creation of novel antibiotics or other treatment modalities. Enhance our knowledge of how different antibiotics interact with bacterial cells, potentially leading to the development of more personalized and tailored treatment regimens. In the end, this research could help patient resistance outcomes, lower antibiotic-resistant healthcare expenditures, and support international efforts to combat the rising threat of antibiotic-resistant diseases. Among the biggest risks to global development, food security, and health are the effects of public health on antibiotic resistance. This study focuses on two commonly used antibiotics and a common disease in order to address a crucial component of this issue. Clinical Relevance, the results of this study would directly affect clinical practice and may result in modifications to the prescription of ciprofloxacin and ceftriaxone, which would eventually improve treatment results. Policy Development, insights gained from this study can inform policy makers and health organizations in developing more effective antibiotic stewardship programs and policies to combat antibiotic resistance.

1.8 DELIMITATIONS

The study investigates the effects of ceftriaxone and ciprofloxacin only, without considering other antibiotics. Bacterial isolates are obtained exclusively from Bindura Provincial Hospital and Shashi Hospital. The study is limited to MRSA and MSSA strains from hospitals in Bindura only and does not cover bacterial strains from other locations and excludes other bacterial species. The study also relies on inhibition zone measurements for antibiotic susceptibility, without using molecular techniques or alternative resistance detection methods.

1.9 LIMITATIONS

This study may be limited by the geographic scope of sample collection, as *Staphylococcus aureus* strains were only obtained from Bindura Provincial Hospital and Shashi Hospital, potentially affecting the generalizability of the findings. Additionally, resource constraints limited the analysis to only two antibiotics ceftriaxone and ciprofloxacin excluding others that may have provided a broader understanding of resistance patterns. The study is conducted within a limited timeframe, which may restrict long-term observations on bacterial resistance patterns. Differences in bacterial resistance mechanisms may introduce variations in inhibition zones that are not accounted for in this study.

1.10 DEFINATION OF TERMS

Antibiotics: Antibiotics are drugs that stop bacteria from growing or kill them in order to treat bacterial infections (AMBOSS, 2024).

Staphylococcus aureus: A frequent gram-positive bacterium on human skin and mucous membranes is *Staphylococcus aureus*. It is a major contributor to sepsis, pneumonia, and skin infections, among other infections. (Wikipedia, 2025)

Ceftriaxone: Ceftriaxone is a wide ranging cephalosporin antibiotic that works by preventing the formation of bacterial cell walls.

Ciprofloxacin: Ciprofloxacin is a fluoroquinolone antibiotic that stops bacteria from growing and surviving by blocking their ability to replicate their DNA.

Growth: Growth refers to the increase in the number of bacterial cells over time, typically measured by colony-forming units (CFUs) or optical density (OD) in bacterial cultures.

Antibiotic Resistance: Resistance in the context of antibiotics refers to the ability of bacteria to withstand the effects of an antibiotic, either through intrinsic mechanisms or acquired resistance genes, leading to reduced susceptibility or complete ineffectiveness of the antibiotic.

Minimum Inhibitory Concentration (MIC): Agar diffusion techniques or broth dilution are frequently used to assess the minimum inhibitory concentration (MIC) of an antibiotic.

Biofilm Formation: Biofilm formation is the process by which bacteria adhere to surfaces and form a complex structure composed of bacterial cells encased in an extracellular matrix, leading to increased resistance to antibiotics.

Antibiotic Susceptibility: Antibiotic susceptibility refers to the degree to which bacteria are susceptible or resistant to the effects of a particular antibiotic, as determined by testing methods such as disk diffusion or broth microdilution.

Mechanisms of Antibiotic Action: Antibiotic mechanisms of action refer to the specific ways in which antibiotics target and inhibit bacterial growth, such as disruption of DNA replication, protein synthesis, and cell wall production, or other essential bacterial processes.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

This chapter reviews existing literature on *Staphylococcus aureus*, its characteristics, and the global challenge posed by antibiotic-resistant strains such as MRSA. It examines the pharmacological properties and mechanisms of action of ceftriaxone and ciprofloxacin, highlighting their documented effects on both susceptible and resistant strains. The review also explores the various mechanisms by which *S. aureus* develops resistance, including enzymatic inactivation, target site modification, reduced drug uptake, and biofilm formation. Finally, it outlines emerging research directions, such as combination therapies, optimized dosing regimens, and molecular investigations of resistance pathways, providing a context and rationale for the current study.

2.2 WHAT IS *STAPHYLOCOCCUS AUREUS*?

A common human skin and nasal canal colonizer that is facultative anaerobic and Gram-positive, *Staphylococcus aureus* frequently does no harm to humans. In contrast, this organism is a noteworthy example of an opportunistic pathogen that can result in a variety of infections, from minor soft tissue and skin infections to more serious illnesses like pneumonia, endocarditis, septicaemia, and osteomyelitis. Surface proteins that encourage colonization, invasions that assist tissue invasion, and toxins that can harm host tissues and elicit immunological responses are only a few of the virulence characteristics that define *S. aureus*. The bacteria *Staphylococcus aureus* is one of the primary reasons of infections linked to hospital settings as well as infections acquired in the community since it has evolved several defence mechanisms against the effects of antibiotics.

Classification provides a systematic framework for identifying and understanding organisms by answering the foundational question. In the context of this study, classification is used to elucidate the biological identity of *Staphylococcus aureus*, guiding our understanding of its structure, function, and clinical relevance. Classification helps define its identity by placing it within a biological hierarchy based on shared characteristics. *S. aureus* belongs to:

Domain: Bacteria, the broadest and most inclusive taxonomic category, indicating that *S. aureus* is a unicellular organism lacking a nucleus but possessing a cell wall. This domain-level classification marks the starting point of the biological hierarchy.

Phylum: Firmicutes, signifying gram-positive bacteria with a thick peptidoglycan layer.

Class: Bacilli, including both rod-shaped and spherical bacteria like Staphylococcus.

Order: Bacillales, a diverse group of gram-positive bacteria.

Family: Staphylococcaceae, comprising cocci commonly found on skin and mucous membranes Patel, Brown, & Gray, (2020).

Genus: Staphylococcus, referring to grape-like clusters of cocci.

Species: *Staphylococcus aureus*, identified by its golden pigment and potential to cause various infections. These features are clinically significant as they aid in laboratory identification and correlate with the bacterium's capacity to are responsible for a variety of illnesses, ranging from minor skin infections to serious systemic disorders.

Domain: Bacteria

Phylum: Firmicutes

Class: Bacilli

Order: Bacillales

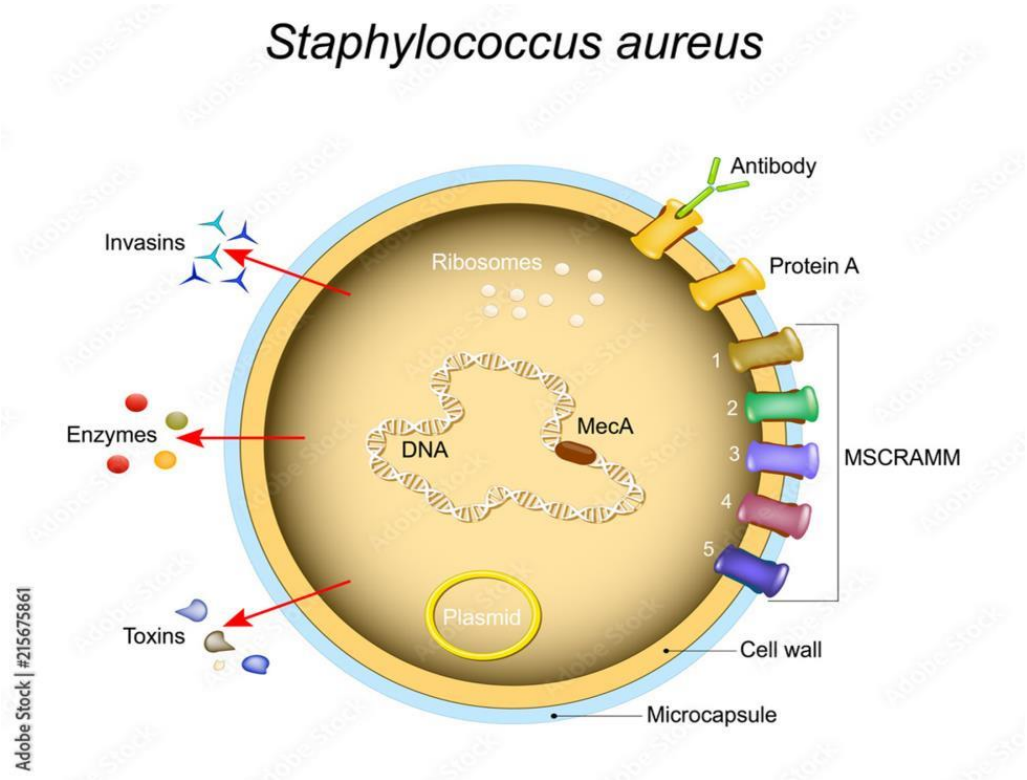
Family: Staphylococcaceae

Genus: Staphylococcus

Species: Staphylococcus aureus

***Staphylococcus aureus* cell structure and pathogenic factors**

Gram-positive cocci with a diameter of 1 μm are called staphylococci. They group together.



(dreamsite.com, n.d, 2008.)

Figure 1: ***Staphylococcus aureus cell structure***

2.3 CHARACTERISTICS OF STAPHYLOCOCCUS AUREUS

Its capacity to aggregate into spherical clusters, or cocci, and to generate a range of variables that increase virulence, like toxins, enzyme, and cell surface proteins, define it. *S. aureus* has the ability to infect the epidermis, respiratory system, and circulation, among other regions of the body. According to Otto (2008), *S. aureus*'s capacity to create biofilms on surfaces increases its resistance to antibiotics and ads to the duration of infections.

The remarkable ability of *Staphylococcus aureus* to develop resistance to multiple antibiotics is a major clinical challenge. MRSA, or methicillin-resistant *Staphylococcus aureus*, is one such instance. It is resistant to all beta-lactam medicines and poses serious treatment difficulties in both community and medical settings. Comprehending the mechanisms underlying *S. aureus* pathogenicity and antibiotic resistance is crucial for formulating efficacious therapeutic approaches and effectively controlling the dissemination of infections engendered by this versatile and frequently hazardous bacterium (Boucher & Corey , 2008)

2.4 EFFECTS OF CEFTRIAXONE AND CIPROFLOXACIN ON *STAPHYLOCOCCUS AUREUS*

Antibiotics exert their effects on bacteria by targeting important cellular processes which include nucleic acid replication, protein synthesis, and cell wall synthesis, and metabolic pathways. These disruptions inhibit bacterial growth or lead to cell death, depending on whether the antibiotic is bacteriostatic or bactericidal. Using its ability to attach to penicillin-binding proteins (PBPs), the third-generation cephalosporin antibiotic ceftriaxone stops bacteria from forming cell walls. Broad-spectrum action of ceftriaxone is demonstrated in opposition to *S. aureus* and other gram-positive and gram-negative colonies. The fluoroquinolone antibiotic ciprofloxacin inhibits DNA replication and transcription by targeting both topoisomerase IV and bacterial DNA gyrase. In clinical contexts, ceftriaxone and ciprofloxacin have both shown effectiveness against *S. aureus* (Dalhoff, 2014).

Recent studies have explored the efficacy of ceftriaxone and ciprofloxacin against *Staphylococcus aureus*, including methicillin-resistant strains (MRSA). Mancl et al. (2018) reported that the combination of ceftriaxone and ciprofloxacin exhibited synergistic activity against MRSA in vitro, suggesting potential benefits of dual therapy in clinical settings. Similarly, Elaraki and colleagues (2019) found that both antibiotics demonstrated strong antimicrobial activity against clinical isolates of MRSA, highlighting their continued relevance in treating resistant infections. However, while these findings are promising, most of the evidence is derived from in vitro studies, and the clinical effectiveness of such combinations particularly against highly resistant or biofilm-forming strains, remains insufficiently explored. There is also a lack of large-scale surveillance data to determine how consistently MRSA strains respond to these antibiotics across different geographic and hospital settings. These gaps underscore the necessity of additional clinical and molecular studies that support these conclusions and direct evidence-based MRSA treatment plans:

Inhibition of bacterial growth: Both ceftriaxone and ciprofloxacin can inhibit the growth of *Staphylococcus aureus* by interfering with essential bacterial processes. Ceftriaxone works by inhibiting bacterial cell wall synthesis, while ciprofloxacin inhibits bacterial DNA replication and synthesis.

Development of antibiotic resistance: According to (Holmes et al, 2016) repeated exposure to these antibiotics can select for resistant strains by promoting genetic mutations or the acquisition of resistance genes, ultimately reducing the efficacy of treatment. Similarly, (Ventola, 2015) emphasized that misuse of broad-spectrum antibiotics like fluoroquinolones and cephalosporin's is a significant driver of resistance in both community and healthcare settings. This can make the bacteria less susceptible to the effects of these antibiotics and more difficult to treat.

Induction of stress responses: Treatment with ceftriaxone and ciprofloxacin can induce stress responses in *Staphylococcus aureus*, leading to changes in gene expression, cell membrane integrity, and other bacterial functions.

Synergistic effects: Ceftriaxone and ciprofloxacin may have synergistic effects when used in combination, enhancing their antibacterial activity against *Staphylococcus aureus*. This combination therapy can help to prevent the development of antibiotic resistance and improve treatment outcomes.

Studies have demonstrated that the synergistic effect of these antibiotic combination can enhance bactericidal activity and reduce the development of resistance in *S. aureus* (Alves, 2020). By targeting different cellular processes and pathways, ceftriaxone and ciprofloxacin can work together to inhibit resistance mechanisms and delay the emergence of resistant. This combination therapy has the potential to provide more effective treatment options for *S. aureus* infections, although challenges in clinical implementation and the evolution of resistance remain areas of concern. Further research is needed to optimize dosing regimens and address potential resistance issues associated with the ceftriaxone-ciprofloxacin combination in *S. aureus* (Galata, 2018).

2.5 MECHANISMS OF ANTIBIOTIC RESISTANCE IN *STAPHYLOCOCCUS AUREUS*

One of *S. aureus's* most concerning characteristics is the emergence of methicillin-resistant strains (MRSA), which are resistant to beta-lactam medications including methicillin, penicillin, and cephalosporins. The main mechanism for resistance to beta-lactam antibiotics is the acquisition of the MecA gene, which results in penicillin binding protein 2a (PBP2a), a protein with a low affinity for these medications, as illustrated in Figure 2 (Chambers & DeLeo, 2009).

Staphylococcus aureus exhibits resistance to multiple classes of antibiotics through a range of mechanisms. While beta-lactam resistance is a major concern largely due to the production of beta-lactamases and modifications to penicillin-binding proteins. *S. aureus* also resists other antibiotics through mechanisms such as increased activity of efflux pumps, which expel antibiotics from the bacterial cell, and changes to target sites that reduce antibiotic binding (Fischbach & Walsh, 2009). Figure 2 illustrates these key mechanisms of antibiotic resistance in *Staphylococcus aureus*:

Enzymatic inactivation: *Staphylococcus aureus* can generate enzymes that can alter or break down antibiotics, making them less effective. Among the most well known enzymes produced by *S.aureus* is beta-lactamase. This enzyme hydrolyzes the beta-lactam ring present in antibiotics such as penicillins and

cephalosporins, rendering them inactive. By producing beta-lactamase, *Staphylococcus aureus* can effectively neutralize the effects of these antibiotics.

Changes to the target sites: *Staphylococcus aureus* can modify the target sites of antibiotics, thereby reducing their effectiveness. One common target for antibiotics such as beta-lactams is penicillin binding proteins (PBPs), which have a role in the production of cell walls. Through the acquisition of mutations in PBPs or the upregulation of alternative proteins, *Staphylococcus aureus* can alter these target sites, making them less susceptible to the inhibitory effects of antibiotics.

Decreased drug uptake: *Staphylococcus aureus* can develop mechanisms to reduce the uptake of antibiotics into bacterial cells, limiting their effectiveness. One such mechanism involves the upregulation of efflux pumps, before the antibiotics can start working, they actively push them out of the bacterial cell.. By increasing the expression of efflux pumps, *S.aureus* can reduce the intracellular concentration of antibiotics, leading to resistance.

Biofilm formation: *Staphylococcus aureus* has the ability to generate complex biofilms structures composed of bacterial cells encased in an extracellular polymer matrix. Biofilms provide a protective environment for bacteria, shielding them from the effects of antibiotics and host immune responses. Bacteria within biofilms are often more resistant to antibiotics compared to planktonic bacteria, making it challenging to eradicate infections caused by biofilm-forming *S.aureus* strains.

Resistance gene acquisition: *Staphylococcus aureus* can get resistance genes via means of horizontal gene transfer, such as metamorphosis, transduction, and conjugation from other bacteria, bactriophages, enviromental DNA and even plasmids. These resistance genes can encode enzymes that degrade antibiotics, efflux pumps that actively pump out antibiotics, or other mechanisms of resistance. The presence of resistance genes in *S.aureus* strains can confer resistance to multiple classes of antibiotics, leading to multidrug-resistant or extensively drug-resistant phenotypes.

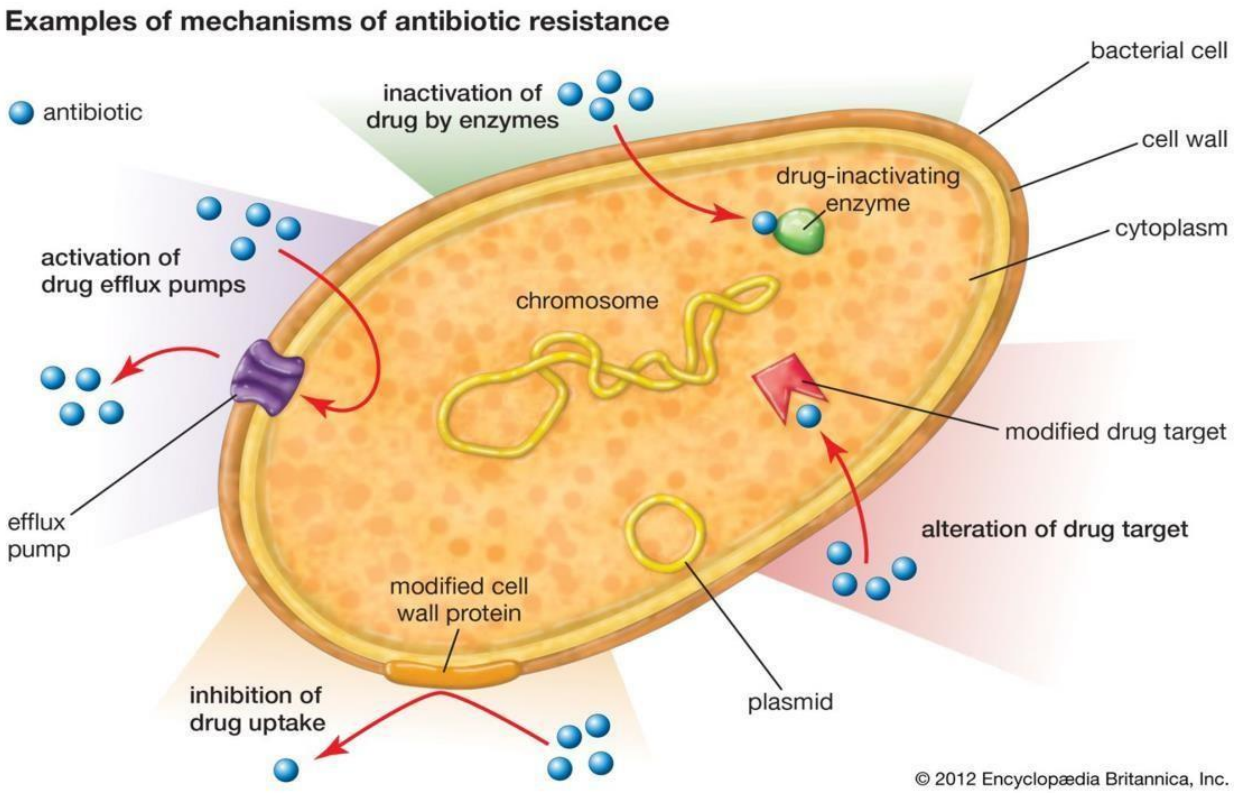


Figure 2: Mechanisms of antibiotic resistance

2.6 FUTURE DIRECTIONS IN ANTIBIOTIC RESEARCH FOR *STAPHYLOCOCCUS* INFECTIONS

The possible clinical ramifications and future directions of the effects of the antibiotic's ceftriaxone and ciprofloxacin, as well as their dosage levels, on *Staphylococcus aureus* growth,

Development, and resistance, must be taken into account. Research has indicated that in order to effectively treat *S. aureus* infections, it is crucial to optimize dosing regimens and investigate combination therapy (McGregor, 2019). To overcome the difficulties presented by *S. aureus* strains that are resistant to antibiotics, it is essential to comprehend the mechanisms of resistance as well as the possible synergistic effects of these drugs. Future studies in this field might concentrate on creating novel drug delivery methods, addressing new resistance mechanisms, and carrying out clinical trials to evaluate the effectiveness of ciprofloxacin and ceftriaxone in various patient populations (Jones, 2020). Researchers can aid in the creation of more focused and efficient treatment plans for S. aureus by carrying out more research on the intricate interactions between antibiotic dose levels and the pathogen's growth.

CHAPTER 3

MATERIALS AND METHODS

3.1 STUDY AREA

The experimental investigation was conducted within a controlled Bindura town of Zimbabwe in microbiology laboratory at environment over a period of seven days.

3.2 SOURCE OF RESEARCH MATERIAL

Bacterial strains, Methicillin Susceptible *Staphylococcus aureus* (MSSA) and Methicillin Resistant Staphylococcus aureus (MRSA), was obtained from Bindura Provincial Hospital and Shashi Hospital. Both susceptible and resistant MSSA isolates were included to facilitate a comprehensive comparative analysis of antibiotic resistance mechanisms. (Smith, 2020). The antimicrobial agents, ceftriaxone and ciprofloxacin, were procured from a certified medical supplier and stored under manufacturer- recommended conditions to preserve their pharmacological integrity.

3.3 RESEARCH DESIGN

The experimental study aimed to evaluate the effects of varying concentration levels of ceftriaxone and ciprofloxacin on the growth and resistance development of *Staphylococcus aureus* using a controlled laboratory setup. The experimental design included multiple treatment groups, replications, and two phases of antibiotic exposure (initial and re-exposure).

3.3.1 **Treatments**

There were four main treatment categories, each with varying antibiotic concentration levels:

Ciprofloxacin-only

Low concentration: **250 mg**

High concentration: **1000 mg**

Ceftriaxone-only

Low concentration: **20 mL** (from 1g powder)

High concentration: **5 mL** (more concentrated solution from 1g powder)

Ciprofloxacin + Ceftriaxone (Combination)

Low concentration: **250 mg** ciprofloxacin + **20 mL** ceftriaxone

High concentration: **1000 mg** ciprofloxacin + **5 mL** ceftriaxone

Controls

Positive control: Standard therapeutic dose (Ciprofloxacin 500 mg + Ceftriaxone 10 mL)

Negative control: No antibiotic applied

In total, **seven (7) treatment groups** were included (3 categories × 2 concentrations + 2 controls).

3.3.2 Replications and Repeats

Each treatment was replicated **2 times** per trial to ensure statistical validity and reliability of results.

The entire experiment was conducted **twice (n=2 independent runs)** on separate days to validate consistency of findings.

3.3.3 Experimental Units

Each Petri dish containing Mueller-Hinton agar and inoculated with either MSSA or MRSA represented a single experimental unit.
A total of:

7 treatments × 2 replications × 2 runs = **28 Petri dishes** were used for the initial exposure phase.

An additional **8** set of dishes was used for re-exposure testing of pre-exposed isolates.

3.3.4 Sampling Method

Bacterial isolates were obtained using purposive sampling from two clinical sources: Bindura Provincial Hospital and Shashi Hospital. Two types of bacterial strains were included: Methicillin Susceptible *Staphylococcus aureus* (MSSA) and Methicillin Resistant *Staphylococcus aureus* (MRSA). For each experimental run, fresh cultures were revived from stored clinical isolates. After the first exposure, select colonies showing growth under low and high antibiotic concentrations were isolated using sterile loops and re-inoculated for the re-exposure phase.

Table 1: ANTIBIOTICS DILUTION CONCETRATION LEVELS

ANTIBIOCS	LOW	HIGH	POSITIVE CONTROL	NEGATIVE CONTROL
CEFTRIAXONE (1g)	**20ml**	**5ml**	**10ml**	**0ml**
CIPROFLOXACIN (20ml saline)	**250mg**	**1000mg**	**500mg**	**0ml**
CEFTRIAXONE& CIPROFLOXACIN	**20ml (cef) + 250mg (cip)**	**5ml (cef) + 1000mg (cip)**	**10ml (cef) + 500mg (cip)**	**0ml**

Ceftriaxone was refrigerated at 2-8°C and shielded from light exposure, whereas ciprofloxacin was stored at ambient temperature (15-25°C) in a moisture-free, light-protected environment (Jones & Patel, 2019). The culture media, comprising Mueller-Hinton agar and nutrient broth, was carefully prepared and sterilized via autoclaving to maintain aseptic conditions. Following incubation, bacterial strains that exhibited inhibition under varying ceftriaxone and ciprofloxacin concentrations were subjected to standard antibiotic dosages to assess their potential for adaptive resistance.

To enable experimental validity and reproducibility, stringent biosafety protocols was upheld throughout the study. Aseptic techniques were rigorously maintained, with all experimental materials sterilized pre- and post-use to mitigate cross-contamination risks. Researchers adhered to standardized laboratory safety procedures, including the utilization of personal protective equipment (PPE) such as gloves, laboratory coats, and eye protection, during all bacterial handling and antimicrobial susceptibility testing. Bacterial cultures and biohazardous materials were disposed of in strict accordance with institutional biosafety guidelines to minimize environmental and health hazards. The study was executed in a strictly controlled laboratory setting to optimize experimental accuracy, facilitate reliable inhibition zone measurement, and enhance the interpretability of antibiotic resistance development trends.

3.4 EXPERIMENTAL PROCEDURE

3.4.1 Preparation of agar:

Mueller-Hinton (MH) agar was prepared from commercially available powdered media, following the MicroMedia Laboratories Pvt. Ltd. manufacturer's instructions provided on the product packaging or datasheet. However, a general preparation method based on standard laboratory protocols, specifically the Clinical and Laboratory Standards Institute (CLSI) guidelines M100, 13st edition (2018) was followed to ensure consistency and accuracy in antibiotic susceptibility testing. The appropriate amount of powder, typically 38 g per litre of distilled water, was weighed according to the manufacturer's specifications. After swirling the powder in distilled water, the mixture was brought to a boil to guarantee full dissolving. To sterilize the medium, it was autoclaved for 15 minutes at 121°C. The medium was autoclaved and then allowed to cool to between 45 and 50°C. Next, a consistent depth of around 4 mm was achieved by pouring it into sterile petri dishes, with roughly 25 mL of medium per 90 mm plate. Before being stored between 2 and 8°C until they were needed, the plates were allowed to harden at ambient temperature.

3.4.2 Preparation of antibiotic concentrations.

Ciprofloxacin

Four **(4)** teaspoons **(20 mL)** of distilled, room-temperature water were poured into three separate small glass containers or bowls. Each bowl labelled as follows: **CIP (½), CIP (1),** and **CIP (2).**

- **CIP (½): 250 mg** ciprofloxacin tablet
- **CIP (1): 500 mg** ciprofloxacin tablet
- **CIP (2): 1000 mg** ciprofloxacin tablet

The respective ciprofloxacin tablets were placed into labelled bowls containing distilled water and left to sit for five (5) minutes until they began to disintegrate. The mixtures were then stirred thoroughly until the tablets were fully dissolved, with no visible residue remaining at the bottom of the bowls. Subsequently, 30 small, disc-shaped paper pieces were cut, about 6mm diameter. Ten discs were labelled according to each antibiotic mixture and soaked in their respective solutions until required for use.

Ceftriaxone

One gram (1g) of ceftriaxone powder was measured and placed into three (3) separate small glass bowls. The bowls were labelled as CEF (A), CEF (B), and CEF (C). Subsequently, 10 ml, 5 ml, and 20 ml of saline or distilled water were added to CEF (A), CEF (B), and CEF (C), respectively. Each solution was mixed thoroughly until the ceftriaxone powder was completely dissolved, ensuring no residue remained at the bottom of the bowls. Thirty small disc-shaped paper pieces were cut, about 6mm diameter. Ten discs were labelled for each respective ceftriaxone dilution (A, B, and C) and were soaked in their corresponding antibiotic solutions until required for use.

Ceftriaxone and ciprofloxacin

The procedures for preparing both ceftriaxone and ciprofloxacin solutions were repeated to obtain soluble mixtures of each antibiotic. These soluble mixtures were then combined into three separate bowls to create strong antibiotic mixtures at their respective concentration levels. The bowls were labelled as MIX (A), MIX (B), and MIX (C). Subsequently, 30 small disc-shaped paper pieces were cut, about 6mm diameter. Ten discs were labelled for each respective mixture (MIX A, MIX B, and MIX C) and were soaked in their corresponding antibiotic solutions until required for use.

3.4.3 Inoculation of Agar Plates:

A frozen culture of Methicillin-Susceptible Staphylococcus aureus (MSSA) was thawed at room temperature or in the refrigerator before being used to inoculate the nutrient agar plates for bacterial growth and subsequent antibiotic susceptibility testing. The nutrient agar plates from section 3.4.1 were divided into sections corresponding to the different treatment groups: ceftriaxone, ciprofloxacin, positive control, and negative control. Using sterile swabs, the bacterial suspension was evenly distributed across each section of the agar plate. The plate was streaked in a zig-zag pattern to promote the formation of isolated colonies. The inoculated plates were left to air dry for approximately 5–10 minutes to ensure the bacterial cells adhered to the agar surface. The agar plates were labelled accordingly with the antibiotic being tested (ceftriaxone or ciprofloxacin) (Alves, 2020).

3.4.4 Placement of Antibiotic Discs

Sterile forceps were used to place antibiotic impregnated discs onto the inoculated agar plates. The discs were pressed gently onto the agar surface to ensure proper contact. Each plate was divided into four segments and received discs containing same antibiotic concentration, ensuring even distribution. The plates were left for 5 to 10 minutes at room temperature to enable antibiotic diffusion before incubation.

3.4.5 Incubation conditions

The inoculated plates were incubated at 37°C in an incubator for 24 hours to allow bacterial growth and antibiotic interaction. The plates were placed in an inverted position to prevent condensation from affecting the bacterial colonies. After incubation, observations were made for bacterial growth and inhibition zones. Any contamination or abnormalities in bacterial growth were noted and accounted for in the analysis.

3.4.6 Selection and Preparation of Pre- Exposed Bacterial Samples

Bacterial colonies that exhibited inhibition in the first exposure phase were identified. Using a sterile inoculating loop, the selected colonies were transferred into four separate Petri dishes. Methicillin-Resistant *Staphylococcus aureus* (MRSA) was added into a separate Petri dish for comparison. Fresh nutrient agar plates were labelled accordingly to track bacterial strains and antibiotic exposure conditions.

3.4.7 Inoculation and Antibiotic Exposure

Using sterile cotton swabs, the standardized bacterial suspensions were streaked evenly onto fresh Muller Hinton agar plates. The plates were allowed to air dry for 5 to 10 minutes to ensure even bacterial adherence. Antibiotic disks containing standard concentrations of ceftriaxone (10 µg/mL) and ciprofloxacin (5 µg/mL) were applied onto the inoculated agar plates. Care was taken to ensure proper placement of the antibiotic disks to prevent overlapping zones of inhibition. The plates were then incubated at 37°C for 24 hours under aerobic conditions.

3.4.8 Data Collection

After incubation, the agar plates were examined for the presence of clear zones around the antibiotic disks. A digital calliper or ruler was used to measure the diameter of the inhibition zones. Bacterial strains exhibiting reduced inhibition zones were identified as showing potential resistance development. The data were recorded and analysed statistically to determine significant changes in bacterial susceptibility.

The diameters of the zones of inhibition for each antibiotic, minimum inhibitory concentration (MIC) values, and resistance patterns were documented. Bacterial growth over time was measured by recording optical density or colony-forming units (CFUs). The results were contrasted with the Clinical and Laboratory Standards Institute's (CLSI) breakpoints to determine the susceptibility of Staphylococcus aureus to ceftriaxone and ciprofloxacin

3.5 STATISTICAL ANALYSIS

The data were analysed using IBM SPSS Statistics version 23. The dataset included inhibition zone diameters (in millimetres) for each *Staphylococcus aureus* strain exposed to varying concentrations of ceftriaxone and ciprofloxacin. A normality test was performed to assess the distribution of these measurements. These measurements were compared with the initial inhibition zones recorded during the first exposure phase. Additionally, correlation analysis was performed to evaluate the relationship between antibiotic concentration and bacterial inhibition. Microsoft Excel was used to generate regression trend models to illustrate inhibition patterns (Jones, 2019).

CHAPTER 4

RESULTS

4.1 INTRODUCTION

This chapter presents the outcomes of the experiments described in Chapter 3, evaluating the effects of varying concentrations of ceftriaxone and ciprofloxacin, individually and in combination, on *Staphylococcus aureus*. Results are organized into two sections: the first reports mean inhibition zones for methicillin-susceptible and methicillin-resistant strains under different treatments, while the second examines changes in susceptibility after pre-exposure to low and high antibiotic concentrations. Statistical analysis using one-way ANOVA and Tukey's HSD tests determines the significance of differences observed, providing insight into both immediate antimicrobial efficacy and potential resistance development.

4.2 INHIBITION MEAN ZONES OF *STAPHYLOCOCCUS AUREUS* GROWTH UNDER DIFFERENT ANTIBIOTIC CONCENTRATIONS

The results of this experiment revealed that all treatments involving ciprofloxacin, ceftriaxone, or their combination produced measurable mean zones of inhibition against *Staphylococcus aureus* as shown in table 2. The highest inhibition was observed with the combination treatment (CIP + CEF), expressing a synergistic interaction between the two antibiotics. In contrast, the lowest inhibition was noted in single antibiotic treatments at low concentrations, particularly ceftriaxone of 6.8mm. The negative control yielded no zone, confirming bacterial viability in the absence of antibiotics, while the positive control confirmed the experimental system's validity. These zones varied significantly depending on the concentration of the antibiotics used. The size of the inhibition zones was positively correlated with +0.89, the concentration of the antibiotic administered. As the dosage increased from low to high, there was a consistent increase in the inhibition diameter, which is indicative of enhanced antimicrobial efficacy.

Table 2: Mean standard deviation Zone of Inhibition (mm) of S. aureus Under Different Antibiotic Concentrations

Antibiotic Treatment	Low Conc. (mm) ± SD	High Conc. (mm) ± SD
Ciprofloxacin	7.2 ± 0.9	19.3 ± 1.5
Ceftriaxone	6.8 ± 0.8	17.8 ± 1.4
Combo (CIP + CEF)	9.1 ± 1.1	23.5 ± 1.9

Table 3: ANOVA Summary for Zone of Inhibition (Initial Exposure)

Source of Variation	SS	df	MS	F-ratio	p-value
Between Groups	428.93	2	214.47	85.78	<0.001
Within Groups	15.00	6	2.50		
Total	443.93	8			

The **F-ratio of 85.78** and **p-value < 0.001** confirm a statistically significant difference among treatment means.

4.2.1 Tukey's HSD Post Hoc Test

Post hoc comparisons using Tukey's HSD test revealed the following significant differences:

- Combo High vs Combo Low: **p = 0.003**
- CIP High vs CIP Low: **p = 0.001**
- CIP High vs Combo High: **p = 0.034**
- CIP High vs Combo Low: **p = 0.002**

These results, linked to the data in **Table 2 and Table 3**, show that higher concentrations, especially in combination, significantly increased the inhibition of *S. aureus*.

4.3 THE INHIBITION MEAN ZONES OF BACTERIA PRE-EXPOSED TO DIFFERENT ANTIBIOTIC CONCENTRATIONS

A follow up phase of the experiment evaluated bacterial colonies that had previously survived exposure to low and high concentrations of antibiotics. These bacteria were re-cultured and subjected to standard therapeutic concentrations of ciprofloxacin and ceftriaxone to assess changes in susceptibility. Results obtained are presented in **Table 4**, which summarises the mean zones of inhibition after re-exposure to standard antibiotic doses for each bacterial sample type..

Table 4: Mean standard deviation Zone of Inhibition after Re-exposure to Standard Antibiotic Dose

Sample Type	**Ciprofloxacin (5 µg/mL) ± SD**	**Ceftriaxone (10 µg/mL) ± SD**
Unexposed Control	21.5 ± 1.0 mm	19.8 ± 1.0 mm
Pre-exposed (High Conc.)	17.2 ± 0.9 mm	15.9 ± 0.9 mm
Pre-exposed (Low Conc.)	12.3 ± 0.8 mm	10.7 ± 0.8 mm
MRSA Reference	7.5 ± 0.5 mm	5.9 ± 0.5 mm

Furthermore, the MRSA reference strain included in the study displayed the highest resistance, evidenced by the smallest inhibition zones (7.5 mm for ciprofloxacin and 5.9 mm for ceftriaxone). This served to validate the model used in this experiment and emphasized the real-world clinical challenge posed by MRSA in both healthcare and community settings (Lee, 2017). Bacterial samples previously exposed to antibiotics displayed reduced inhibition zones upon re-exposure, indicating a decreased susceptibility and possible development of resistance. The lower the concentration of the initial exposure, the greater the reduction in inhibition during re-testing. These results support the hypothesis that prolonged or repeated antibiotic exposure, especially at non-lethal concentrations, fosters the emergence of resistant bacterial subpopulations. Notably, MRSA showed the smallest inhibition zones, further highlighting the severity of resistance in clinical settings.

A second ANOVA evaluated whether initial antibiotic exposure affected bacterial resistance.

Table 5: ***ANOVA Summary for Post-exposure Inhibition Zones***

Antibiotic	**SS Between**	**df**	**MS Between**	**F-ratio**	**p-value**
Ciprofloxacin	201.12	3	67.04	95.77	<0.001
Ceftriaxone	180.42	3	60.14	98.73	<0.001

Significant p-values demonstrate that the zone of inhibition decreased notably based on exposure type, confirming resistance development. These statistical findings affirm the significance of antibiotic concentration and prior exposure in influencing bacterial susceptibility. The Pearson correlation coefficient (r = -0.86) further highlighted a strong inverse relationship between previous antibiotic exposure and inhibition zone size, indicating that repeated sub-therapeutic exposure diminishes antimicrobial efficacy and fosters resistance.

CHAPTER 5

DISCUSSION, SUMMARY, RECOMMENDATIONS AND CONCLUSIONS

5.1 DISCUSSION

5.1.1 Overview of Key Findings

The study revealed that increasing concentrations of ceftriaxone and ciprofloxacin led to larger zones of inhibition, indicating stronger suppression of *Staphylococcus aureus* growth, while lower concentrations were associated with reduced efficacy and potential for resistance development. The results demonstrated that both antibiotics, either individually or in combination, have the potential to suppress the growth of *S. aureus*, with increased concentrations generally yielding greater zones of inhibition. These findings align with existing literature, which consistently shows that antibiotic efficacy is concentration dependent, and effectiveness can vary significantly with dosage, exposure time, and application method (Dalhoff, 2014; Elaraki, 2019).

5.1.2 Comparative Antimicrobial Efficacy

The inhibition zones recorded in this experiment were consistent with expected antimicrobial behaviour. Ciprofloxacin exhibited a strong inhibitory effect against *S. aureus*, followed closely by ceftriaxone. The most pronounced suppression, however, was observed in the combination treatment (ciprofloxacin + ceftriaxone), suggesting a synergistic interaction between the two antibiotics. This synergistic relationship is likely due to their differing but complementary modes of action: ciprofloxacin disrupts bacterial DNA gyrase and topoisomerase IV (Brown, 2020), thereby inhibiting DNA replication, while ceftriaxone binds to penicillin-binding proteins and interferes with the synthesis of the bacterial cell wall (Garcia, Martinez, & Perez, 2018).

5.1.3 Resistance Development Following Sub-lethal Exposure

One of the significant findings of this study was the impact of prior antibiotic exposure on bacterial resistance. Repeated exposure of *S. aureus* to sub-lethal concentrations of the antibiotics led to a marked reduction in susceptibility during subsequent exposures. This was evidenced by the decreased size of inhibition zones following re-exposure. This result underscores the danger of low dose of antibiotic therapy, which may not eliminate the pathogen but instead encourage the

selection of resistant phenotypes (Jones & Patel, 2019). Such phenotypes may employ a range of adaptive mechanisms, including alterations in gene expression, activation of pumps for efflux, and reduction in membrane permeability, and enhancement of biofilm production (White, 2021).

5.2 SUMMARY

5.2.1 Clinical Implications and Antibiotic Stewardship

This study draws attention to the clinical importance of ensuring that antibiotics are administered at effective therapeutic doses. Insufficient antibiotic dosing, often caused by self-medication, poor adherence to treatment protocols, or substandard drug formulations, can facilitate resistance development (Patel, Brown, & Gray, 2020). Once resistance emerges, it can compromise treatment efficacy and increase the risk of complications, prolonged hospital stays, and higher healthcare costs. The results of this study provide strong evidence for the need to implement proper diagnostic procedures and sensitivity testing before initiating antibiotic treatment. Such steps can assist medical professionals in choosing the right antibiotic and dosage, thus reducing the chances of resistance emergence.

5.2.2 Impacts of Combination Therapy

The study reveals the advantages of combination antibiotic therapy. The use of both ciprofloxacin and ceftriaxone in tandem produced the largest inhibition zones, suggesting that such a strategy can be more effective in certain clinical scenarios. Combination therapies not only offer enhanced antimicrobial activity but can also mitigate the emergence of resistance by attacking bacteria through multiple mechanisms simultaneously. This approach could be particularly beneficial in treating infections caused by multidrug-resistant organisms where monotherapy may be inadequate (Smith, 2020).

5.2.3 Mechanisms of Bacterial Adaptation

The reduced susceptibility observed in pre-exposed bacterial samples reflects the capacity of *S. aureus* to rapidly adapt under selective pressure. These adaptations may involve various biochemical and genetic changes, such as mutations in target enzymes, production of protective biofilms, or modification of metabolic pathways (Gülay et al., 2023). As bacteria develop these defence strategies, the challenge of treating infections intensifies. Therefore, understanding how

such resistance mechanisms evolve is essential for developing next-generation antibiotics or alternative therapies.

5.2.4 Broader Implications and Future Direction

Overall, this research adds to the increasing amount of evidence emphasizing the importance of precise antibiotic use. Thoughtful dosing strategies, backed by laboratory-based sensitivity data and robust statistical analysis, are fundamental in preventing the rise of resistance. Additionally, the observed synergy between ciprofloxacin and ceftriaxone supports the continued investigation into combination therapies as a tool for maximizing treatment outcomes and suppressing bacterial adaptation.

The continued rise of resistant *Staphylococcus aureus* strains, particularly MRSA, necessitates an integrated approach involving healthcare professionals, policymakers, researchers, and the public. Regular resistance surveillance, public health education, and policy enforcement on antibiotic use are crucial in this effort. Future studies should expand on the findings of this research by investigating the molecular mechanisms underlying resistance in *S. aureus* after antibiotic exposure. Genomic, proteomic, and transcriptomic analyses could shed light on specific genes or pathways involved in resistance, offering new targets for drug development. Comparative studies including a wider range of antibiotic classes and bacterial isolates from various clinical sources would further validate these findings and guide global strategies in combating antibiotic resistance.

5.2.5 Limitations of the Study

This study was limited by several factors. Firstly, the number of bacterial isolates used was rather small, which would limit how far the results can be applied. Additionally, the investigation was carried out under controlled laboratory conditions, which do not fully mimic the complex environment of human infection sites. Furthermore, the duration of exposure and the frequency of re-exposure to antibiotics were restricted by time constraints, and more extended exposure periods may yield different results.

5.3 RECOMMENDATIONS

This study recommends the implementation of strict antibiotic stewardship programs in healthcare institutions to regulate the prescription and usage of antibiotics, thereby preventing under-dosing

or misuse that contributes to the development of resistance. Based on the observed synergistic effects of ciprofloxacin and ceftriaxone, clinical practitioners should consider the use of combined antibiotic therapy where appropriate to enhance treatment outcomes and reduce resistance rates. There is a need to support further research into the molecular basis of antibiotic resistance in *S. aureus*, particularly through whole-genome sequencing and molecular analysis to identify resistance genes and mutations arising from repeated antibiotic exposure. Future studies should also expand the scope by including additional bacterial strains and a broader range of antibiotics, which would help in generalizing the findings and assessing resistance trends across various pathogens. Evaluating the long-term effects of antibiotic exposure is essential, and prolonged observational studies are necessary to track how bacterial resistance evolves over time. Incorporating advanced diagnostic techniques, such as molecular and biochemical assays, alongside traditional inhibition zone measurements, will further improve the accuracy and reliability of resistance detection. Public education and healthcare worker training should be prioritized to increase knowledge of the risks associated with self-medication and the significance of finishing prescribed antibiotic courses. These combined efforts can significantly contribute to mitigating the threat posed by antibiotic-resistant *Staphylococcus aureus* and strengthen the global fight against antimicrobial resistance.

Future research should consider a broader range of antibiotic classes, exposure times, and clinical isolates to enhance the validity of these findings. Studies employing molecular techniques such as gene sequencing, expression profiling, and bioinformatics analysis could identify specific resistance genes and their regulatory mechanisms. There is also a need for research on alternative therapies, including bacteriophage therapy, antimicrobial peptides, and plant-derived compounds, to supplement or replace traditional antibiotics in the fight against resistant bacteria.

5.4 CONCLUSIONS

This study provided valuable insights into the impact of varying concentrations of ceftriaxone and ciprofloxacin antibiotics on the growth and resistance development of *Staphylococcus aureus*. It established that both antibiotics exhibited antibacterial activity against *S. aureus*, with the combined use of ciprofloxacin and ceftriaxone demonstrating a stronger synergistic effect than when either antibiotic was used alone. The findings confirmed that the effectiveness of antibiotics

is concentration-dependent, with higher doses producing more significant zones of inhibition. Furthermore, the results indicated that repeated exposure of *S. aureus* to sub-lethal concentrations of antibiotics led to reduced susceptibility upon subsequent treatments, supporting the claim that inappropriate antibiotic usage can facilitate resistance development. The study also demonstrated that MRSA strains remain highly resistant to conventional antibiotics, further underlining the urgent need for more effective antimicrobial management strategies. The outcomes of this research support the hypothesis that exposure to varying antibiotic concentrations impacts both the growth and resistance dynamics of *Staphylococcus aureus*. By linking antibiotic dose levels to bacterial suppression and resistance patterns, the study contributes to a better understanding of how to optimize antibiotic therapy to prevent the emergence and spread of resistant strains. The findings from this study underline the complexity of antibiotic-bacteria interactions and the critical importance of appropriate antibiotic use in managing infections caused by *Staphylococcus aureus*. Effective treatment protocols must consider not only the immediate efficacy of antibiotics but also their long-term impact on resistance patterns. Addressing these challenges through research, education, and policy implementation is vital in safeguarding the future of antimicrobial therapy.

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LIST OF APPENDICES