Chapter One

Introduction

1.1 Background of the Study

Resistance among bacteria to antimicrobial drugs has been brought on by the widespread use of antibiotics, as well as the non-compliance, inappropriate treatment, and overprescribing that are associated with it, as well as poor infection prevention and control. Antimicrobial resistance (AMR) is one of the biggest challenges in modern medicine, with the emergence of multi-drug resistant bacteria posing a significant threat to public health. This issue prompts us to explore for new tactics in the fight against bacterial infection. Several regions of Nigeria have reported cases of antibiotic resistance. In 2009, reports were made on the rapid evolution of flouroquinolone-resistant *Escherichia coli* in a Nigerian community as well as the establishment of a community-associated methicillin-resistant *Staphylococcus aureus* in southwest Nigeria[1,2]. The common Gram-positive and Gram-negative isolates from various clinical specimens in a tertiary hospital in Nigeria also had significant antibiotic resistance rates[3]. According to reports, one of the major risk factors for antibiotic resistance is widespread drug overuse. A significant proportion of antibiotic intake was found in this group of Nigerian university students, showing the knowledge and practices in the use of antibiotics[4]. The authors pointed out that they frequently buy their antibiotics from unreliable sources without a doctor's prescription and do not finish their prescribed course of treatment. Parallel to this, a survey of a few villages in Jos, Nigeria, revealed that consumers have little awareness about antibiotics and unfavourable views toward their use[5].

According to a Nigerian scenario study in 2017, a combined data from the Federal Ministries of Health, Environment, and Agriculture stated that 20% of all *Neisseria meningitidis* isolates were resistant to chloramphenicol and 30.8% were resistant to penicillin G. This is based on

data from five meningitis studies. Penicillin G, chloramphenicol, and ceftriaxone resistance rates for *Haemophilus influenzae* were reported to be 66.7%, 23.5%, and 4.5%, respectively, and to be 45.2%, 10.3%, and 1.7%, respectively, for *S. pneumoniae*[6].

According to an interview with Adefolarin Opawoye, an infectious disease expert at an hospital, he stated that drugs like ciprofloxacin and augmentin no longer work on patients infected by organisms that produce extended spectrum beta lactamases which means that you have to move on to more powerful substitutes such as piperacillin-tazobactam and meropenem[6].

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a particularly concerning pathogen, responsible for a wide range of infections, including skin and soft tissue infections. MRSA has developed resistance to multiple antibiotics, making it increasingly difficult to treat. Compared to patients infected with non-MRSA, those with Skin infections caused by MRSA have a higher risk of bacteremia, hospital re-admission, and death and frequently need longer and more expensive periods of hospitalization[7]. According to European recommendations, MRSA infections should be treated with vancomycin, teicoplanin, linezolid, daptomycin, tigecycline, or ceftaroline; nevertheless, reports of antibiotic resistance have already been made[8]. Novel antimicrobial drugs and approaches are thus urgently needed to combat MRSA in Skin and Soft Tissue Infections.

Bacteriocins, produced by various bacteria, are a diverse group of antimicrobial cationic and hydrophobic peptides composed of 20-60 amino acids that exhibit potent activity against a range of bacterial pathogens, including MRSA[9]. The ribosomal machinery is responsible for the production of bacteriocins. Several genes are thought to play a role in self-immunity proteins, bacteriocin export and control, and amino acid modification[10]. The genes that produce bacteriocins are arranged into operons that can be found on chromosomes, plasmids,

or other mobile genetic elements, and these operons are often inducible and need bacterial peptides to be secreted and accumulated extracellularly in order to be activated[11,12]. They differ from most therapeutic antibiotics due to their limited activity spectrum and proteinaceous makeup. They are extracellularly released and are characterized by a bactericidal or bacteriostatic action against strains of the same species or closely related species[13]. Bacteriocins' antibacterial efficacy against many bacteria, fungi, parasites, viruses, and even against naturally resistant formations such bacterial biofilms is a crucial characteristic[14]. They are defined as a heterogeneous group with bactericidal action on various types of bacteria and are considered a promising alternative to traditional antibiotics due to their narrow spectrum of activity and low potential for resistance development.

Lactic acid bacteria (LAB) are a variety of Gram-positive bacteria. They are categorized based on the features of glucose fermentation, cell morphology, ability to absorb sugars, and optimal development temperature range. They are core group made comprised of the genera *Leuconostoc*, *Lactobacillus*, *Pediococcus*, and *Streptococcus*[15]. The number of genera have increased according to molecular biological techniques which include: *Aerococcus*, *Alloiococcus*, *Carnobacterium*, *Dolosigranulum*, *Enterococcus*, *Lactococcus*, *Lactosphaera*, *Melissococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella*[15]. Several studies have demonstrated that LAB has a probiotic effect, degrades mycotoxins, and inhibits the growth of pathogenic bacteria[16]. LAB are widely distributed in nature and interact symbiotically with higher organisms. They have been isolated from several natural sources such as dairy products, meat, fruits, and vegetables, among others. They can also be discovered in plants, wastewater, soil, and manure, as well as in the mucous membranes of the respiratory, gastrointestinal, and other anatomical areas of both humans and animals. Due to their fermentation of food raw materials, Lactic Acid Bacteria (LAB) are responsible for a

significant variety in the flavor and texture of food products. However occasionally they may to be blamed for food spoiling[17].

Combination therapy, using two or more antibiotics, has been used for many years to treat bacterial infections. The rationale for combination therapy is to increase the efficacy of treatment and reduce the potential for resistance development. Combining natural antimicrobials with antibiotics may help to lower the dosage of individual medications, reduce adverse effects, stop the development of drug resistance, and remove resistant strains[18]. To prevent bacterial resistance, the prospect of replacing the use of antibiotics is researched. According to numerous studies, LAB bacteriocins are effective against *Listeria monocytogenes* and *Staphylococcus aureus* and have synergistic effects with other biomolecules like Nisin and Citric acid[19]. Also, it is known that bacteria can become resistant to bacteriocins. Yet, compared to traditional antibiotics, bacteriocin resistance is quite low. Given that bacteriocin-exposed cells seldom undergo spontaneous mutations[18].

This research aims to investigate the synergistic action between bacteriocins and antibiotics against resistant strains of some bacteria such as MRSA and bacteria implicated in skin infections. The study will involve in vitro assays to determine the activity of bacteriocins and antibiotics individually and in combination against MRSA and other bacterial pathogens and opportunistic pathogens associated with skin infections.

1.2 Statement of the Problem

The emergence of antibiotic-resistant bacteria, such as Methicillin-resistant *Staphylococcus aureus* (MRSA), has become a major public health concern worldwide. MRSA is commonly associated with skin infections, which are difficult to treat due to its resistance to traditional antibiotics, such as Penicillin. Drug resistance is responsible for an estimated 700,000 deaths

annually worldwide and AMR is predicted to cause over 10 million deaths annually and more than $100 trillion USD in lost production globally by 2050 if current trends continue[20].

Antibiotics have been used to treat infections successfully for a long time. They also make the management of infectious diseases easier, which reduces morbidity and mortality. Recent research, however, indicates that the development of antimicrobial resistance (AMR) in both hospital and community settings threatens the benefits brought about by antibiotics[21]. Standard treatment is no longer effective due to this recent trend, which complicates patient management and raises patient morbidity and mortality[22].

Drugs like Ciprofloxacin, Augmentin, Methicillin and Penicillin no longer work on patients infected by organisms that produce extended spectrum beta lactamases, which means concentration has to be moved on to prolonged therapy with more powerful substitutes such as Piperacillin-tazobactam, Vancomycin and Meropenem. However, prolonged therapy with these substitutes have very serious side effects.

The alarmingly high rate of treatment resistance among diseases necessitates the creation of novel medications. New medication research is a time-consuming and expensive process, moreover, microorganisms quickly acquire resistance to new medications. This may reduce the anticipated commercial life cycle (of the medications), making it less appealing for the major pharmaceutical corporations to invest in the discovery and development of new antibiotics.

Few studies have looked specifically at how antibiotics and non-antibiotic antibacterial agents interact to combat various infections. But there are still fewer research on the combined impact of Lactic acid bacteria and antibiotics on pathogenic bacteria. Therefore, this research aims to evaluate the potential synergistic action between bacteriocins and traditional antibiotics against MRSA and resistant bacteria associated with skin infections and

determine the combination with the best synergestic effect, which could provide a new approach for the treatment of these infections.

1.3 Justification of the Study

MRSA and some other Skin and Soft Tissue Infections (SSTIs) can cause significant morbidity and mortality, particularly in immunocompromised individuals, children, and the elderly. The development of new antibiotics to combat these infections has been slow, and the overuse and misuse of antibiotics have led to the emergence of antibiotic-resistant strains of bacteria. Bearing in mind that combination therapy of antibiotics is already failing, it is pertinent to try out other methods of therapy such as combining bacteriocins with antibiotics. These anti-microbial agents may enhance the activity of each other against MRSA and other resistant skin infective bacteria.

Bacteriocins being an organic substance is capable of maintaining its activity such that bacteria do not acquire resistance against them easily. Also, combining bacteriocins with traditional antibiotics may enhance the efficacy of both against MRSA and other bacteria-causing skin infections. Furthermore, this combination therapy may reduce the likelihood of development of antibiotic resistance. Bacteriocins have been shown to be effective against a wide range of bacterial pathogens, including MRSA. However, their use as therapeutics is limited due to their narrow spectrum of activity and low stability.

Combining bacteriocins with antibiotics, such as penicillin, may enhance their activity against MRSA and other bacteria associated with bacterial skin infections. This is because the two agents may have a synergistic effect, with each agent enhancing the activity of the other. Furthermore, the combination therapy may reduce the likelihood of the development of antibiotic resistance.

There is already some evidence to suggest that the synergistic action of bacteriocins and antibiotics is effective against MRSA and other bacteria associated with skin infections. For example, a study published in the World Journal of Pharmaceutical research found that the combination of the bacteriocin from *Cronobacter sakazakii*, and antibiotics was more effective against *S. aureus* than either agent alone[23]. Another study published in Frontiers of immunology found that the combination of the thiopeptide Microccin p1, and Rifampicin was effective against MRSA in a murine skin infection model[24].

The use of combination therapy with bacteriocins and antibiotics, such as penicillin, may be an effective strategy to combat antibiotic-resistant bacteria associated with skin infections. Further research is needed to optimize the use of these agents in combination and to evaluate their effectiveness in clinical settings.

1.4 Aims and Objectives of the Study

The study aims to evaluate the potential of crude bacteriocin in an in vitro testing as a treatment strategy for skin infections caused by Methicillin-resistant *Staphylococcus aureus* (MRSA), Candida and other bacteria.

The study also aims to do an in vitro investigation of the synergistic action of bacteriocin and antibiotics against MRSA, Candida and other clinically important pathogenic bacteria implicated in skin infections.

The objectives of this research are to:

i. Isolate and characterize Lactic Acid Bacteria (LAB) from natural sources such as fresh cow milk.
ii. Extract bacteriocins from LAB.

iii. Determine by in vitro investigation the activity of known antibiotics against MRSA and other clinically important pathogenic bacteria implicated in Skin infections.

iv. Evaluate the antimicrobial activity of the combination of bacteriocin and antibiotics against MRSA and other bacteria implicated in skin infections.

1.5 Research Questions

1. What is the comparative efficacy of combining bacteriocins and antibiotics versus using each treatment separately in addressing skin infections caused by antibiotic-resistant bacteria?
2. What is the spectrum of activity of bacteriocin-antibiotic combinations against different bacterial species associated with skin infections, and how does it compare to conventional antibiotics?
3. Can this synergistic therapeutic approach be adapted to specific skin infection scenarios, such as diabetic foot ulcers or methicillin-resistant Staphylococcus aureus (MRSA) infections?
4. What are the challenges and potential limitations in the production, formulation, and administration of bacteriocin-antibiotic combinations for clinical use in skin infection treatment?
5. What is the spectrum of activity of bacteriocin-antibiotic combinations against different bacterial species associated with skin infections, and how does it compare to conventional antibiotics?
6. Can the synergistic therapeutic approach involving bacteriocins and antibiotics help reduce the development of antibiotic resistance in skin infection-causing bacteria?

1.6 Significance of the Study

The significance of studying the potential of bacteriocins and antibiotics as synergistic therapeutic agent against MRSA and bacteria associated skin infections is multi-fold.

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Firstly, bacterial infections caused by MRSA and other bacteria are becoming increasingly difficult to treat due to the rise of antibiotic resistance. This poses a significant threat globally, as it limits the effectiveness of currently available antibiotics, increases the risk of exposure to extreme side effects of other available powerful antibiotic alternative and increases the risk of complications and mortality associated with bacterial infections.

Therefore, the use of bacteriocins in combination with antibiotics may provide a potential solution to this problem, given that bacteriocin resistance is quite low. Also, bacteriocin-exposed cells seldomly undergo spontaneous mutations[25]. By combining bacteriocins with antibiotics such as vancomycin, the synergistic effect may enhance the overall antibacterial activity, reducing the likelihood of antibiotic resistance development and reduce the possibility of extreme side effects.

Secondly, skin infections are a common site for bacterial infections, and *Staphylococcus* and *Streptococcus* are frequent causative agent. The use of a synergistic approach between bacteriocin and antibiotics may prove to be a promising alternative therapy for skin infections caused by these bacteria.

Therefore, the significance of studying the potential synergistic action between this two antimicrobials against MRSA and pathogenic bacteria associated skin infections lies in the potential to overcome the growing problem of antibiotic resistance, improve the potency of treatment, and provide alternative therapeutic options for the management of bacterial infections.

1.7 Scope of the Study

This study will test the synergy of two antimicrobials (bacteriocins and antibiotics) against some selected bacterial strains isolated from infected skins through in vitro experiments. The bacterial strains to be tested will include MRSA as well as other common bacteria associated with skin infections such as *Staphylococcus aureus*, *Pseudomonas sp*, and *Escherichia coli, Raoultella ornithinolythica* etc. The effect of the combination on bacterial growth will also be investigated.

1.8 Limitation of the Study

There are several limitations and challenges to studying the potential synergy of bacteriocin and antibiotics against pathogenic and resistant strains. The following are a few that should be considered when designing and interpreting such study:

1. There are limited clinical data on the efficacy of bacteriocins in treating skin infections caused by resistant bacteria. Clinical trials involving bacteriocins are not as extensive as those for traditional antibiotics, making it challenging to establish their safety and effectiveness in humans[26].
2. The use of antibiotics, even in combination with bacteriocins, may still contribute to the development of antibiotic resistance. This is a significant concern when considering the long-term implications of this treatment approach[27].
3. Bacteriocins are typically highly specific to certain bacterial strains because of their narrow spectrum. This means that their efficacy may vary depending on the strain of bacteria causing the skin infection. There may also be concerns about the potential for the development of resistance against bacteriocins in the target bacteria[28].
4. Bacteriocins may also not be effective against all types of skin infections. Their narrow spectrum of activity may be limited to certain Gram-positive bacteria, and

they may not be effective against Gram-negative bacteria, which are responsible for many skin infections[20].

5. Some bacteriocins can have toxic effects on human cells and may trigger allergic reactions. Therefore, assessing the safety profile of bacteriocins is essential, especially when considering their use in skin infections[10].
6. Determining the exact mechanisms of synergy between bacteriocins and antibiotics can be challenging. The interactions between these two agents may vary based on the specific antibiotic and bacteriocin used, making it challenging to generalize findings[29].
7. The in vivo efficacy can also be a limitation to this study. Research on the synergistic effect of bacteriocins and antibiotics often relies heavily on in vitro studies, and translating these results to in vivo situations can be complex. Factors such as pharmacokinetics, bioavailability, and host immune response may impact the real-world efficacy of this combination therapy[30].
8. The practicality of using bacteriocins as part of a therapeutic regimen for skin infections may be limited by production costs and regulatory hurdles. The development and approval of bacteriocin-based therapies may be more time-consuming and costly compared to traditional antibiotics[31].
9. Also, the response to bacteriocin-antibiotic therapy may vary among individual patients due to factors such as age, underlying health conditions, and the microbiome, and this variability can make it challenging to predict treatment outcomes[32].
10. Lastly, the use of bacteriocins may raise ethical concerns, such as sourcing and production methods, as well as the potential environmental impact of large-scale bacteriocin production[33].

1.9 Operational Definition of Terms

Bacteriocin: Bacteriocins are proteinaceous compounds, produced by bacteria, which have antimicrobial activity against other bacteria, particularly strains that are closely related.

Antibiotics: Antibiotics are pharmacological agents that inhibit the growth or kill bacteria. Antibiotics refer to synthetic or natural compounds that are commonly used in medical practice to treat bacterial infections.

Lactic Acid Bacteria: Lactic acid bacteria (LAB) are a group of bacteria that convert sugars into lactic acid through the process of fermentation. They are commonly found in various natural environments, including dairy products, plants, and the human digestive system.. Some well-known LAB species include Lactobacillus, Streptococcus, and Bifidobacterium.

Synergistic Therapeutic Agent: A synergistic therapeutic agent is a combination of two or more substances that, when used together, have a greater therapeutic effect on the target condition than the individual agents used alone.

Resistant Strains of Bacteria: Resistant strains of bacteria are bacterial populations that have developed mechanisms to withstand the inhibitory or killing effects of antibiotics, rendering them less susceptible to standard antibiotic treatment.

Pathogenic Bacteria: Pathogenic bacteria are a type of microorganism that can cause diseases in humans, animals, and plants. These bacteria possess virulence factors, which are characteristics or mechanisms that enable them to invade host tissues, evade the host's immune response, and produce toxins or other harmful substances. When pathogenic bacteria infect a host, they can lead to various infectious diseases, ranging from mild illnesses to severe and life-threatening conditions.

Skin and Soft Tissue Infections: Skin and soft tissue infections refer to a range of conditions involving bacterial overgrowth or colonization on or within the skin, causing various dermatological issues such as cellulitis, abscesses, impetigo, and others.

Endnotes

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Chapter Two

Literature Review

2.1. Bacteriocin

Bacteriocins are ribosomally generated antimicrobial peptides with minimal toxicity and high antibacterial activity at clinically appropriate concentrations. They are typically stable over a wide range of temperature and pH[1]. They are antimicrobial peptides or proteins, which can be considered as safe since they can be effortlessly broken down by proteolytic enzymes of the mammalian gastrointestinal tract. Likewise, most bacteriocin producers belong to Lactic acid bacteria (LAB), a group that takes place normally in food varieties and have a long history of safe use in dairy industry. Since they represent no wellbeing risk concerns, bacteriocins, either refined or discharged by bacteriocin-producing strains, are an extraordinary option in contrast to the use of substance additives.

A few bacteriocins have shown a definite mode of action in contrast to regular antibiotics, which might diminish the possibility of cross-resistance improvement, permitting them to be considered as promising options in contrast to antibiotics. For instance, the chance of resistance development by lanthipeptides, e.g, Nisin is diminished, because of their different modes of action and the pyrophosphate moiety nature of their objective (which isn't normal in regular antibiotics)[2]. Besides, mixture of bacteriocins and different antimicrobials or antibiotics with various mechanisms of action might expand their antimicrobial strength while lessening the chance of resistance development[3,4]. Many examinations have shown the inhibitory impacts of various bacteriocins against microbes behind hospital-acquired infections e.g, Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Enterococcus*, *Clostridium difficile* and numerous Gram-negative pathogenic microbes, such as, *Moraxella catarrhalis*, *Neisseria spp*. and, *Haemophilus influenza*[5]. For example,

microcin J25, a Gram-negative bacteriocin, was displayed to show a high antimicrobial action against multidrug-resistant *Salmonella* and *E. coli*[6]. Also, bacteriocins can be utilized as inhibitors of multidrug-resistant *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter spp*. and, New Delhi Metallo-beta-lactamase-1 (NDM-1) indicating *Enterobacteriaceae*. Bacteriocins have been fundamentally read up in vitro for their viability against clinically significant microorganisms; accordingly, they can be prospective applicant for treatment of infectious diseases, like those influencing the oral, respiratory, gastrointestinal and urogenital tracts. Sanitized and microbiologically specified bacteriocins, for example, mutacin 1140 and salivaricin D, have shown high inhibitory actions against strains liable for respiratory diseases in people, specifically *Streptococcus pneumoniae*, *S. aureus* and *P. aeruginosa*. Additionally, nisin F was accounted for to display in vitro and in vivo inhibitory actions against clinical strains of *S. aureus*[7]. There are likewise a few reports on the productivity of bacteriocins against bacteria in charge of gastric ulcers, and skin infection. Besides, effective use of bacteriocins has been declared to be effectively tested for skin infection, oral sicknesses and mastitis in breastfeeding women. What's more, bacteriocin treatment has been productively utilized as an option in contrast to antibiotics in treating *C. difficile* infections[8].

The narrow spectrum of some bacteriocins expects that the strains answerable for a disease should be recognized before treatment with bacteriocins can begin, that is, preventing their utilization to treat infections of unspecified etiology. Nevertheless, this attribute restricts the probability of reactions on the natural healthy microbiota[9]. For example, antibiotic treatment of *C. difficile* causes antibiotic incited interruption of gut microbiota, permitting the microorganism to regrow and cause *C. difficile*-associated diarrhoea (CDAD). In any case, the sactibiotic thuricin CD, which is produced by a strain of *Bacillus thuringiensis*, is a narrow spectrum bacteriocin that targets *C. difficile*. Thuricin CD has shown high

antimicrobial action that is similar to that of vancomycin and metronidazole, but varies by virtue of having limited effects on the commensal microbiota of the gut. More examples include pediocin PA-1, which effectively treated *L. monocytogenes* infection in mice without seriously altering commensal, and subtilosin A, which inhibits *Gardnerella vaginalis* without affecting *Lactobacillus spp*[10].

2.1.1 Updated Classifications of Bacteriocins

For the previous years, a several classifications of bacteriocins have been proposed by Klaenhammer[11]. As of late, to characterize novel bacteriocins, Alvarez-Sieiro proposed a changed arrangement scheme in view of the biosynthesis mechanism and biological activity in accordance with different recommendations[12]. Bacteriocins of lactic acid bacteria, of which there are a lot, alone have been classified in different ways, prompting two to four subclasses. These classifications have been changed commonly based on new improvements in regards to structures and modes of action of bacteriocins[13].

According to the classification illustrated by Cotter, Ross and Hill, which is clear and straightforward and applying to bacteriocins from both Gram-positive and Gram-negative microscopic organisms, bacteriocins were mostly sorted into two major classes in light of the presence or not of post-translationally altered themes. In addition, this order put into consideration just antimicrobial peptides and not larger antimicrobial proteins, for example, colicins from *Escherichia coli*[14].

In view of these guidelines and considering recent improvements on ribosomally integrated and post-translationally adjusted peptides (RiPPs), we propose here a refreshed classification of bacteriocins from Gram-positive and Gram-negative microbes in three large classes: Class I – small post-translationally modified peptides; Class II – essentially unmodified

bacteriocins; and Class III – larger peptides (>10 kDa, thermo-labile), being each one subdivided into subclasses.

Class I gathers peptides with atomic masses <5 kDa that all contain PTMs guaranteed by dedicated enzymes encoded in the bacteriocin gene cluster. These class I bacteriocins in this way belong to the group of RiPPs.

Class II bacteriocins are basically unmodified peptides of 6-10 kDa and including or not balancing out disulfide bridges. The adjustment make class I bacteriocins more steady to high temperatures, extreme pHs or proteolytic proteins than class II ones, despite the fact that disulfide bridges likewise increase the class II peptide steadiness. Class I is further branched following the RiPP classification. This includes lanthipeptides, sactipeptides, round peptides and glycocins from Gram-positive bacteria[14], linear azole (ine) - containing peptides (LAP) and lasso peptides from both Gram-positive and Gram-negative microorganisms, and nucleotide peptides and siderophore peptides from Gram-negative bacteria.

Class III bacteriocins are huge, heat-labile (>10 kDa) protein bacteriocins. This class is subdivided in two subclasses: subclass IIIa (bacteriolysins) and subclass IIIb. Subclass IIIa contains those peptides that kill bacterial cells by cell wall degeneration, in this manner causing cell lysis. The best researched bacteriolysin is lysostaphin, a 27 kDa peptide that hydrolyzes the cell wall of a few *Staphylococcus* species, primarily *S. aureus*. Subclass IIIb, conversely, includes those peptides that don't cause cell lysis, killing the target cells by obstructing plasma membrane potential.

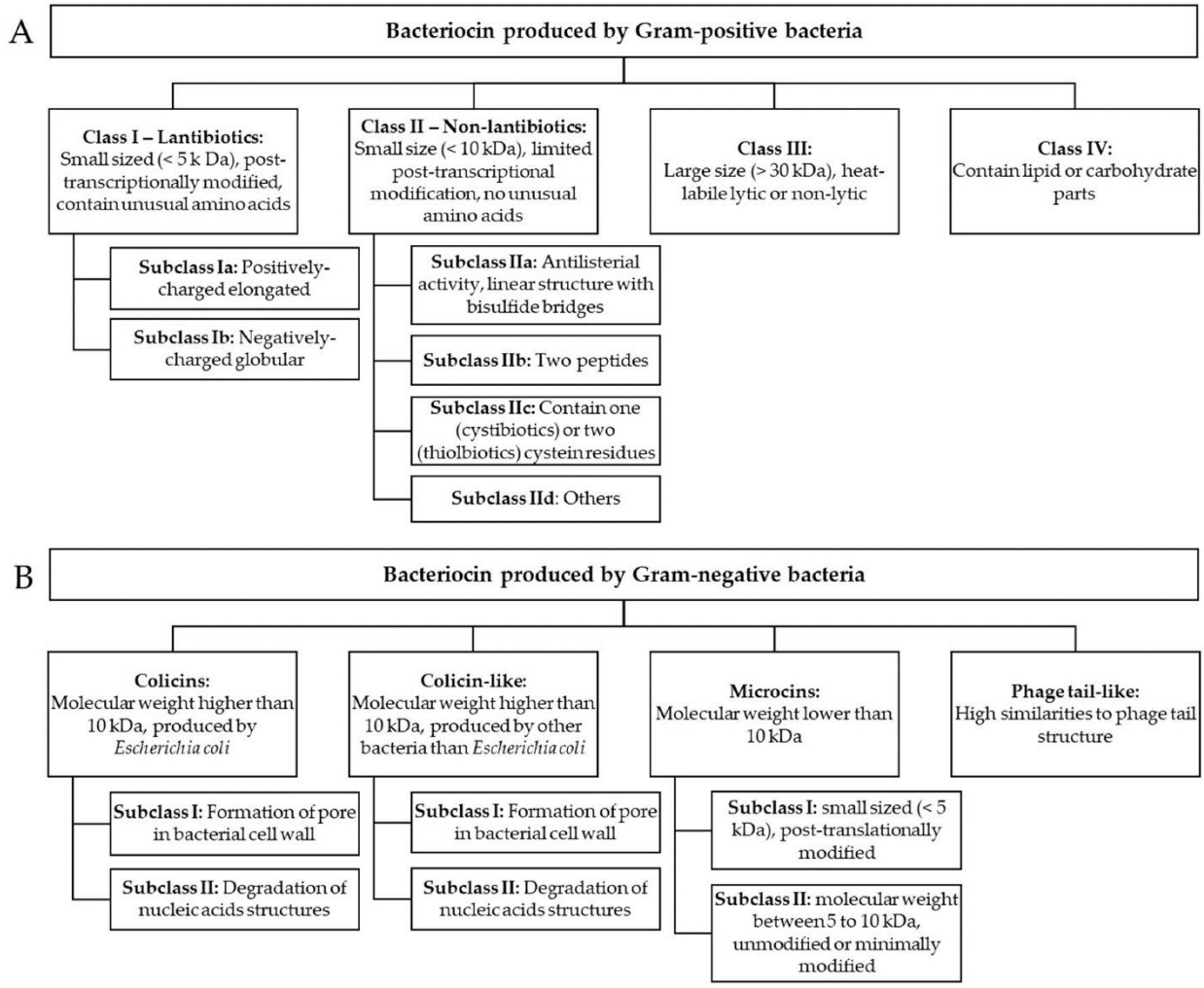

Figure 2.1: Classification of bacteriocins produced by Gram-positive (A) and Gram-negative (B) bacteria.

Source[14]

2.1.2 Mechanisms of Action of Bacteriocins

Bacteriocins use a variety of ways to prevent the growth of their target organisms. These mechanisms can be loosely classified into two groups: (i) those that regulate gene expression and protein synthesis largely within the cell and (ii) those that primarily act on the cell envelope. Several bacteriocins function by targeting the cell membrane, particularly those that are known to inhibit Gram-positive bacteria. Certain class I bacteriocins prevent the production of peptidoglycan by inhibiting lipid-II on the cell membrane. For their target bacteria to be inhibited or killed, other bacteriocins produce holes (Fig 2). For instance, class II bacteriocins like lactococcin A attach to the Mannose Phosphotransferase System, which forms pores (Man-PTS)[14].

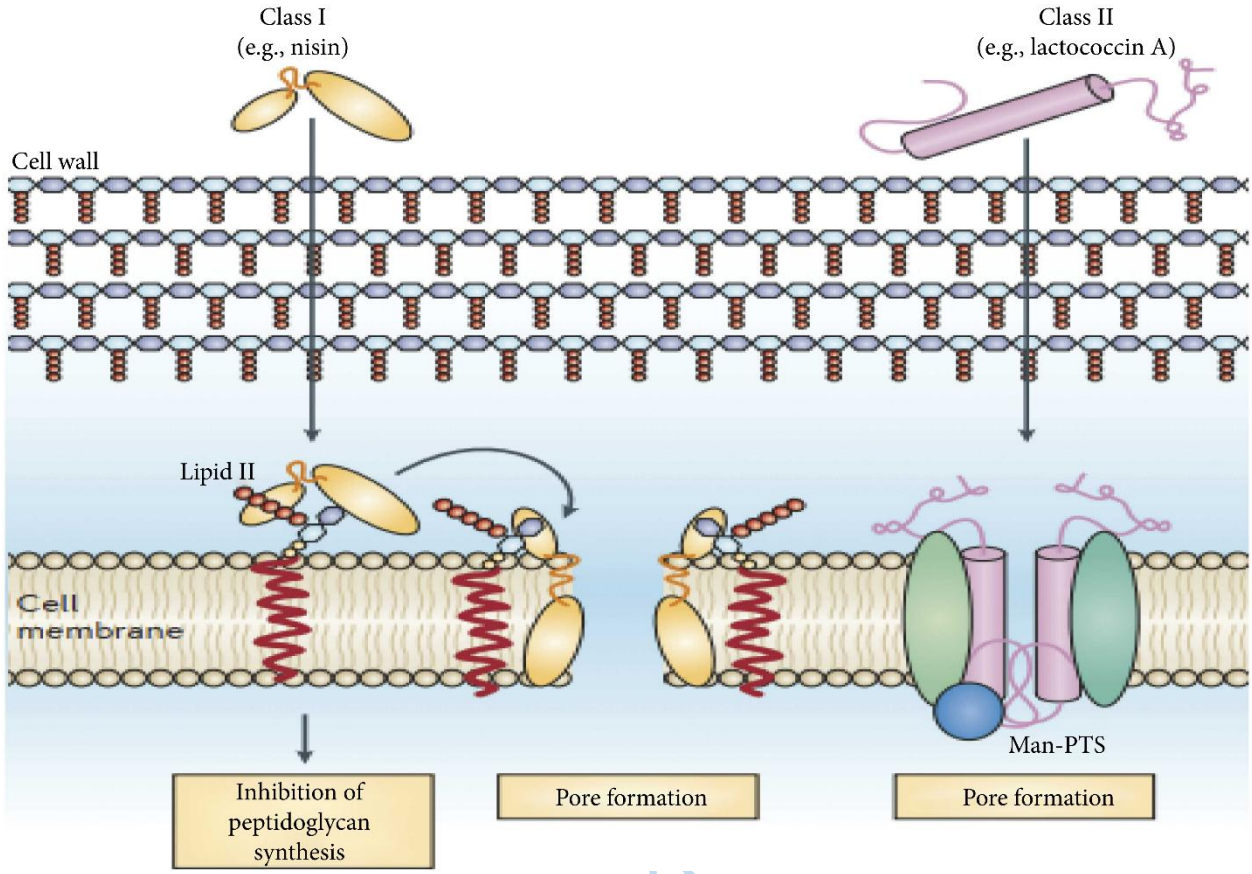

Figure 2.2: Mechanism of Action of Bacteriocins on Gram-positive Bacteria

Source[14]

Several class I or lantibiotic bacteriocins, like nisin, have been demonstrated to have a dual mode of action. They have the ability to attach to lipid-II, the primary carrier of peptidoglycan components from the cytoplasm to the cell wall, preventing proper cell wall production and ultimately causing cell death. Also, they can start a process of membrane insertion and pore formation that results in accelerated cell death by using lipid-II as a docking molecule. Mersacidin only has the lipid-II binding activity but does not create pores, whereas a lantibiotic with two peptides, as lacticin 3147, can have these dual activities distributed over two peptides[15].

Many bacteriocins that inhibit Gram-negative bacteria do so by interfering with the metabolism of DNA, RNA, and proteins. As a result, they must pass through the outer and, in many cases, the inner membranes in order to work. Examples include the inhibition of RNA polymerase by MccJ25, DNA gyrase by MccB17, and aspartyl-tRNA synthetase by MccC7-C51. Yet, there are certain outliers, such as MccE492, which creates pores in order to function[14] (Fig 3). Through their enzymatic actions, several bacteriocins exhibit antimicrobial activity. Megacin A-216 has phospholipase activity against the target organism, while colicin E2 and E3 exhibit DNase and RNase activity, respectively.

The amphiphilic helical nature of class II peptides in general enables them to bind to the target cell's membrane, causing depolarization and death. The cell wall of Gram-positive targets, however, can be directly affected by big bacteriolytic proteins like lysostaphin (class III bacteriocins), which causes the target cell to lyse and die[15].

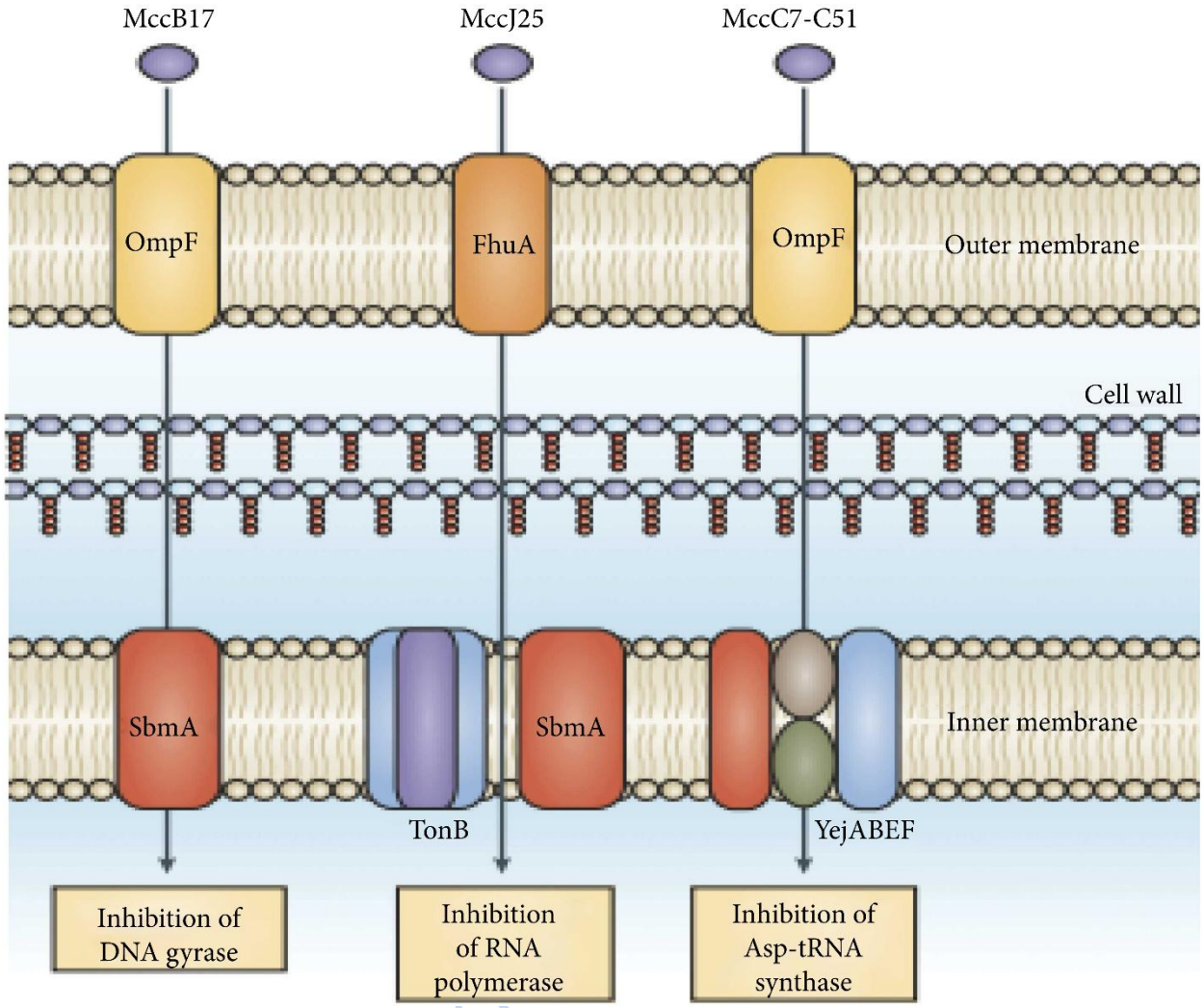

Figure 2.3: Mechanism of Action of Bacteriocins on Gram-Negative Bacteria

Source[14]

2.2 Antibiotics

Antibiotic is a chemical compound that is toxic to other bacteria and is produced by a living organism, usually a bacterium. In a complex environment like soil, organisms undoubtedly use antibiotics to regulate the growth of competing microbes. Antibiotics are frequently produced by soil microorganisms. The bacteria and fungi are microorganisms that produce antibiotics that are helpful in preventing or treating disease. After the invention of penicillin in 1941, antibiotics gained widespread recognition. Since then, they have fundamentally changed how bacterial infections in humans and other animals are treated. Yet, they are ineffectual against viruses.

2.2.1 Mechanisms of Action of Antibiotics

The effects of antibiotics are produced by a number of different modes of action. Several of these drugs, which are typically referred to as β-lactam antibiotics, function by preventing the formation of bacterial cell walls. In order to create the bacterial cell wall, the wall's constituent parts must first be partially assembled inside the cell, then transported through the cell membrane to the developing wall, assembled into the wall, and ultimately cross-linked. Antibiotics that prevent the cell wall from forming have a particular impact on one or more phases. The organism's cell wall and shape change as a result, and the bacteria eventually perishes.

Additional antibiotics that prevent protein synthesis in bacteria include aminoglycosides, chloramphenicol, erythromycin, and clindamycin. Animal and bacterial cells produce proteins in essentially the same ways, however the proteins involved are different. These variations are used by antibiotics that are specifically harmful to attach to or disrupt the activity of bacterial proteins, stopping the production of new bacterial cells and proteins.

Antibiotics like polymyxin B and polymyxin E (colistin) attach to phospholipids in the bacterial cell membrane and prevent it from acting as a selective barrier, allowing vital macromolecules to flow out and causing the cell to die. These antibiotics are relatively hazardous since other cells, including human cells, have comparable or identical phospholipids.

Certain antibiotics, like the sulfonamides, compete with one another to prevent the synthesis of folic acid (folate), a crucial precursor to the synthesis of nucleic acids. Sulfonamides can prevent the formation of folic acid because they resemble the intermediary substance (para-aminobenzoic acid), which is transformed into folic acid by an enzyme. Due to their similar chemical structures, para-aminobenzoic acid and sulfonamide are in competition for the enzyme that transforms the intermediate into folic acid. Reversing this reaction by taking away the chemical causes the bacteria to be inhibited but not killed.

By attaching to a component on the bacterial enzyme in charge of RNA duplication, the antibiotic rifampin prevents bacteria from synthesizing ribonucleic acid (RNA). Rifampin has a significantly larger affinity for the bacterial enzyme than it does for the human enzyme, therefore at therapeutic levels it has no effect on human cells.

2.2.2 Categories of Antibiotic

The spectrum of activity of antibiotics—specifically, whether they are narrow-, broad-, or extended-spectrum agents—can be used to classify them. Gram-positive bacteria are largely affected by narrow-spectrum antibiotics like penicillin G. Tetracyclines and chloramphenicol are examples of broad-spectrum antibiotics that have an impact on both Gram-positive and certain Gram-negative bacteria. An antibiotic with an extended-spectrum affects new bacterial species, often Gram-negative ones, as a result of chemical alteration. In order to distinguish between bacteria with cell walls made of a thick meshwork of peptidoglycan [a

peptide-sugar polymer] and bacteria with cell walls that only have a thin layer of peptidoglycan, the terms Gram-positive and Gram-negative are used.

2.2.3 Major Antibiotics

Each type of antibiotic has a particular role in medicine and can be used as a useful model to examine the numerous ways that antibiotics work. The antibiotics used to treat tuberculosis, Imipenem, Penicillins and Cephalosporins, as well as the drugs Aztreonam, Bacitracin, and Vancomycin, are covered in the sections that follow. These substances and subgroups of substances serve as more examples of the chemical and functional variety seen in antibiotics.

Penicillins

The β-lactam ring, a special structural feature of the penicillins, is what gives them their potent antibacterial properties. The final stage in the construction of the cell wall is accomplished by the bacterial cell's proteins through interactions with the β-lactam ring.

Penicillins can be split into two categories: semisynthetic penicillins and naturally occurring penicillins (penicillin G, penicillin V, and benzathine penicillin). Growing the mold Penicillium under conditions where just the fundamental molecule (6-aminopenicillanic acid) is produced allows for the production of semi-synthetic penicillins. This molecule can be modified to produce a variety of semi-synthetic penicillins that differ in their resistance to the enzyme -lactamase (penicillinase), which selectively breaks the β-lactam ring, rendering the antibiotic inactive.

The preferred medications for treating meningococcal infections, tonsillitis caused by some *Streptococci*, endocarditis, syphilis, and streptococcal sore throats are still the naturally occurring penicillins. Penicillinase-resistant penicillins were created as a result of several bacteria, most notably *Staphylococcus*, developing resistance to naturally occurring penicillins (methicillin, oxacillin, nafcillin, cloxacillin, and dicloxacillin). Methicillin is no

longer utilized due to the advent of Methicillin-Resistant *Staphylococcus aureus* (MRSA), which has severely restricted the use of numerous of these medicines.

Cephalosporins

The penicillins' mechanism of action is the same as that of the cephalosporins. The core chemical structures of penicillins and cephalosporins, however, differ in various ways, which causes some variation in the antibacterial activity spectrum. Four generations of cephalosporins were created by altering the fundamental chemical (7-aminocephalosporanic acid) produced by *Cephalosporium acremonium*.

The broad-spectrum penicillins previously mentioned share some antibacterial activity with the first-generation cephalosporins (cefazolin, cephalothin, and cephalexin). For example, they work well against the majority of *staphylococci*, *streptococci*, and penicillin-resistant *pneumococci*.

The second-generation cephalosporins, which include cefamandole, cefaclor, cefotetan, cefoxitin, and cefuroxime, offer a wider range of antibacterial action, including increased activity against more Gram-negative rod species. As a result, these medications work to combat the bacteria *Escherichia coli*, *Klebsiella*, and *Proteus species* (though several strains of these organisms have developed resistance). While cefoxitin is especially active against the majority of *Bacteroides fragilis* strains, cefamandole is also effective against a number of *Haemophilus influenzae* and *Enterobacter* infections. However, the activity of second-generation cephalosporins against gram-positive bacteria has diminished.

In comparison to second-generation drugs, the third-generation cephalosporins (ceftriaxone, cefixime, and ceftazidime) are more active against gram-negative pathogens. These medications are effective against the majority of *Enterobacter* species, as well as *H. influenzae* and several *Neisseria* species. The fourth-generation compounds' (cefepime's)

antibacterial range is comparable to that of third-generation medicines, but the fourth-generation medicines exhibit greater resistance to β-lactamases.

The cephalosporins are similarly generally benign to penicillins. Those who are hypersensitive to penicillin may experience hypersensitivity reactions due to the cephalosporins' structural resemblance to that of penicillin.

Aztreonam, Bacitracin, and Vancomycin

Aztreonam is a synthetic antibiotic that inhibits the formation of cell walls and has some built-in resistance against β-lactamases. Aztreonam must be administered parenterally despite having a low incidence of toxicity. A unique strain of the bacteria *Bacillus subtilis* produces bacitracin. Its usage is restricted to the topical treatment of skin infections caused by *Streptococcus* and *Staphylococcus* as well as for eye and ear infections due to its extreme toxicity to kidney cells.

The antibiotic vancomycin, which is made by *Streptomyces orientalis*, is typically administered intravenously because it is poorly absorbed from the gastrointestinal tract. It is used to treat severe *staphylococcal* infections produced by strains that are resistant to different penicillins. The development of vancomycin-resistant *Staphylococcus aureus* was caused by its usage against MRSA (VRSA).

Imipenem

Imipenem is a beta-lactam antibiotic that inhibits the formation of cell walls. Most β-lactamases cannot hydrolyze it because of its high resistance. The gastrointestinal tract cannot absorb this antibiotic, so it must be administered intravenously or intramuscularly. As an enzyme found in the renal tubule hydrolyzes imipenem, it is always given with cilastatin, an inhibitor of this enzyme. The use of imipenem has been constrained by neurotoxicity and seizures

2.2.4 Antibiotic Resistance

The potential for bacterial resistance to antibiotics has long been a worry for antibiotic therapy. A few bacteria that are genetically more resistant to the effects of the antibiotic may survive, despite the fact that an antibiotic may kill almost all of the bacteria causing an illness in a patient. They continue to procreate or pass on their resistance to other members of their species through gene exchange processes. These resistant bacteria spread because antibiotics have eliminated or drastically reduced the number of their more susceptible competitors. Humans eventually develop bacterial infections that are resistant to one or more of the antibiotics that are typically effective in such situations. The spread of such bacterial resistance is encouraged by the indiscriminate and imprecise use of antibiotics.

To combat antibiotic resistance, scientists are always searching for novel antibiotics. Several bacterial toxins and antimicrobial peptides have been identified as potentially useful substances. New therapy approaches, such as combining antibiotics that work synergistically to kill more bacteria, are also being researched. It might be possible to inject substances into bacterial populations that effectively make the bacteria more susceptible to currently available antibiotics[16].

2.3 MRSA (Methicillin Resistant *Staphylococcus Aureus*)

MRSA, or methicillin-resistant *Staphylococcus aureus*, is a bacterial infection that is contagious. Because it is resistant to multiple antibiotics, people occasionally refer to it as a superbug, making treatment a challenging feat because of this resistance. MRSA is a widespread infection that can be potentially serious and has become resistant to many different kinds of antibiotics. Methicillin and related antibiotics like penicillin, vancomycin, and oxacillin are included. An antibiotic related to penicillin is methicillin. It was once effective against the bacterium *Staphylococci* (staph).

Staph bacteria have become resistant to methicillin and other drugs related to penicillin over time. Methicillin is no longer an effective treatment for MRSA, however this does not imply that the infection cannot be treated. It can be treated with some antibiotics. The majority of MRSA infections affect patients who have visited hospitals or other healthcare facilities, like nursing homes and dialysis facilities. Health care-associated MRSA is the term used to describe it when it appears in these settings (HA-MRSA). Invasive operations or equipment, such as surgeries, intravenous tubing, or prosthetic joints, are frequently linked to HA-MRSA infections. Health care professionals who touch patients or patients who touch contaminated surfaces can spread HA-MRSA.

In the larger community, among healthy individuals, another kind of MRSA infection has been reported. Community-associated MRSA (CA-MRSA) frequently starts as an uncomfortable skin boil. Usually, skin-to-skin contact is how it spreads. Those who live in crowded quarters, child care workers, and high school wrestlers are examples of at-risk populations. Since patients with SSTIs caused by Methicillin-resistant *S. aureus* (MRSA) have a higher risk of bacteremia, hospital re-admission, and death, and frequently need longer and more expensive periods of hospitalization than patients infected with non-MRSA, Methicillin-resistant *S. aureus* (MRSA) is of particular concern[17]. According to European recommendations, MRSA infections should be treated with vancomycin, teicoplanin, linezolid, daptomycin, tigecycline, or ceftaroline; nevertheless, reports of antibiotic resistance have already been made[18].

2.3.1 Symptoms

MRSA and other staph skin infections typically begin as painful, bloated red lumps that resemble pimples or spider bites. Possible affected areas may be:

- Red, inflamed and painful

- Warm to the touch
- with excessive pus or other discharge
- coupled with a fever

These red bumps have a rapid capacity to develop into painful, deep boils (abscesses) that call for surgical drainage. The bacterium can occasionally only be found on the skin. Yet, they can also penetrate the body deeply and cause infections that could be fatal in the bloodstream, heart valves, lungs, joints, bones, and surgical wounds.

Serious MRSA infections in the blood or deep tissues can present with the following symptoms:

- A fever of at least 100.4 °F and chills
- Malaise, disorientation, dizziness, muscular pain
- Swelling and soreness in the affected body part
- A chest ache
- Breathlessness and cough
- Headache, rash, and slow wound healing

2.3.2 Causes

The bacteria *Staphylococcus aureus*, or "staph," come in a variety of forms. About one-third of people typically have Staph bacteria on their noses or on their skin. Even then, they often only cause minor skin issues in healthy individuals because the bacteria are mostly harmless unless they enter the body through a cut or other wound. The MRSA strain of staph bacterium is persistently carried by around 5% of the population, according to the Centers for Disease Control and Prevention.

2.3.3 Antibiotic Resistance

Antibiotic usage that was frequently unwarranted over many years led to MRSA. For colds, the flu, and other viral infections that don't react to these medications, doctors have been prescribing antibiotics for years. Antibiotics may not completely eradicate the microorganisms they target, so even when they are used correctly, they contribute to the spread of drug-resistant microorganisms. Because bacteria evolve quickly, pathogens that survive treatment with one antibiotic quickly pick up the ability to resist others[17].

2.4 Bacterial Skin Infections

A remarkable barrier against bacterial infections is provided by the skin. Despite the fact that many bacteria come into contact with or live on the skin, they typically cannot cause an infection. When bacterial skin infections do arise, they can affect any part of the body, whether it be a small patch or the full surface. They may be innocuous or potentially fatal, depending on their severity.

When microorganisms penetrate the skin through hair follicles or tiny skin breaks through scrapes, punctures, surgeries, burns, sunburn, animal or insect bites, wounds, or pre-existing skin conditions, bacterial skin infections can develop. By engaging in a range of activities, such as gardening in polluted soil or swimming in a contaminated pond, lake, or ocean, people may get bacterial skin infections. While some infections just affect the skin, others can affect the soft tissues that are beneath the surface. Among the relatively mild infections are:

2.4.1 Erythrasma

Erythrasma is an infection of the epidermis caused by *Corynebacterium minutissimum*. Most people with erythrasma are adults, particularly those with diabetes and those who live

in hot, muggy locations. The foot is where erythrasma most frequently occurs, when it results in scaling, cracking, and skin breakdown between the last two toes. Also common in the groin, this infection creates tiny scaling and irregularly shaped pink or brown patches, especially where the thighs touch the scrotum (in men). This infection is more common in patients with diabetes and obese middle-aged women, especially in the armpits, skinfolds underneath the breasts or on the belly, and the region immediately in front of the anus (perineum). In some individuals, the infection progresses to the torso and anal area.

2.4.2 Folliculitis

A tiny skin abscess called a folliculitis affects the hair follicle. Without usually involving a hair follicle, other abscesses can develop on the skin's surface as well as within the deeper layers of the skin. *Staphylococcus aureus* bacteria are the most common cause of skin abscesses, which manifest as pus-filled pockets on the skin's surface. A type of *Staphylococcus* that was once susceptible to drugs has recently increased in frequency. Methicillin-resistant *Staphylococcus aureus* is the name of this strain (MRSA).

Even while there is frequently no evident point of entry, the bacteria can occasionally enter the skin through a hair follicle, a minor scrape, or a puncture. Individuals who live in cramped quarters, have poor hygiene habits, suffer from chronic skin disorders, or have *Staphylococcus* in their nasal passages are more likely to experience bouts of folliculitis or skin abscesses. Other prevalent risk factors include immune system deterioration, obesity, advanced age, and possibly diabetes. For reasons that are unknown, certain persons may get recurrent infections. Those who are prone to recurrent infections may be given instructions by doctors to wash their entire body with antibacterial soap, put antibiotic ointment inside their noses where the bacteria can hide, and take antibiotics orally in an effort to get rid of *Staphylococcus*.

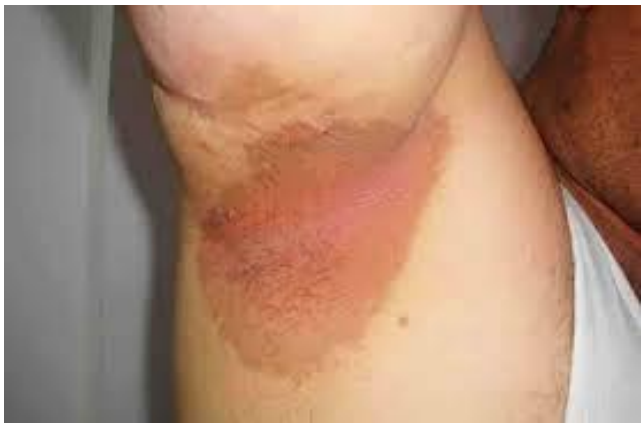

Plate 2.1: Characteristic Pink-brown Patches of Erythrasma in and surrounding the Skinfolds of the Armpit.

Source[19]

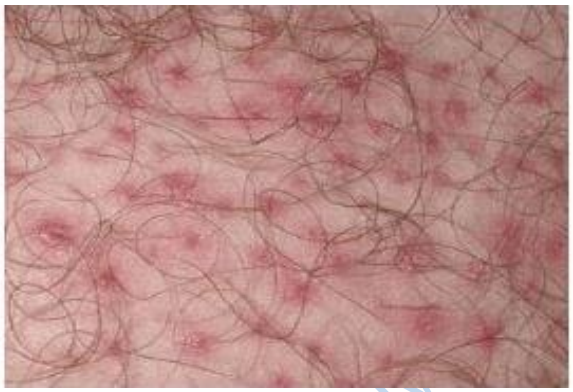

Plate 2.2: Infected and Inflamed Hair Follicles caused by a Bacterial Infection.

Source[20]

Plate 2.3: Clusters of Ruptured Sores Developing a Honey-coloured Crust around the lips of a Young Boy Indicating Impetigo.

Source[21]

2.4.3 Impetigo & Ecthyma

Staphylococcus aureus, *Streptococcus pyogenes*, or both can cause impetigo, a superficial skin infection that causes scabby sores with yellow crusts and, occasionally, tiny blisters that ooze yellow fluid. Impetigo in the form of ecthyma results in sores deeper in the skin. Impetigo is prevalent. Children are primarily affected. Although impetigo can affect any part of the body, it most frequently affects the face, arms, and legs.

Bullous impetigo is one type of impetigo that results in various-sized blisters that can linger for days to weeks. Impetigo frequently affects healthy skin, but it can also happen after an injury or a condition like a fungal infection, sunburn, or insect bite that breaks the skin. Risk factors may include a damp environment and poor hygiene. Some people have *Streptococci* or *Staphylococci* in their noses, but these bacteria don't infect them.

They're referred to as nasal carriers. Those that carry the germs but do not exhibit any symptoms from it are considered carriers. Carriers can transport bacteria from their nose to other body regions by touching them with their hands, which can sometimes result in recurrent infections or infection of others. Impetigo spreads quickly to other people as well as to other parts of the body.

Ecthyma: Little, shallow ulcers with a punched-outlook and occasionally pus-filled are the hallmark of ecthyma. The crust that forms around ulcers is thicker than the impetigo-related crust. It has a brown-black hue. Often, the ulcers' surrounding skin is reddish-purplish and puffy.

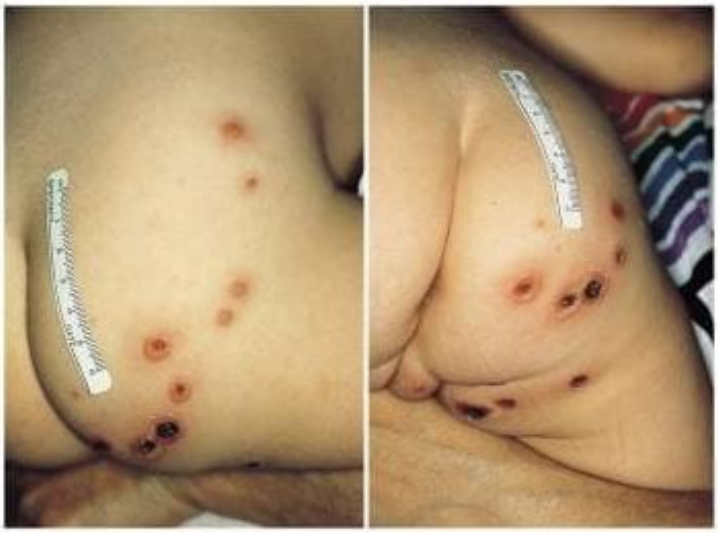

Plate 2.4: Ecthyma showing Small, Pus-containing, Shallow, Punched-out Sores (ulcers) with Thick, Brown-black Crusts.

Source[20]

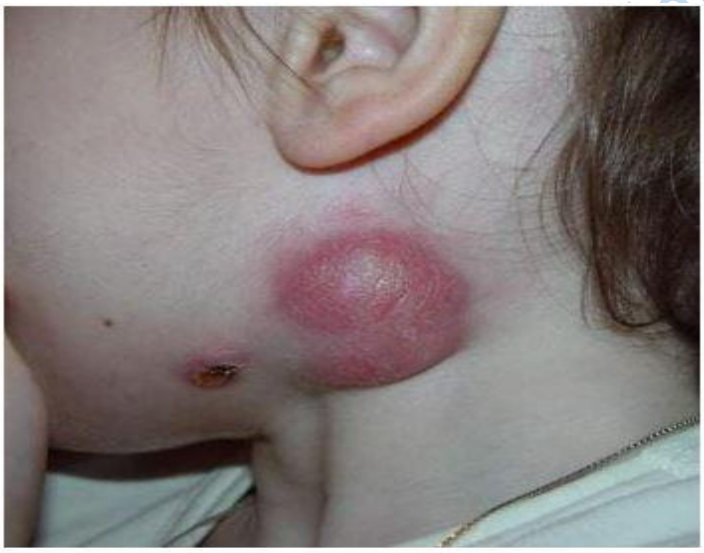

Plate 2.5: Swollen lymph nodes (lymphadenitis) in a child.

Source[20]

2.4.4 Lymphadenitis

One or more lymph nodes can contract lymphadenitis, which results in their swelling and tenderness. Lymph is a fluid that seeps out of the body's smallest blood vessels. The fluid travels between cells, nourishes them, and removes unhealthy cells, cancerous cells, and contagious microbes. All lymph travels through lymphatic channels to lymph nodes that are located in strategic locations. Damaged cells, cancerous cells, and foreign substances are removed from the lymph fluid via lymph nodes. In lymph nodes, specialized white blood cells engulf and kill damaged cells.

Almost always, an infection—which could be brought on by bacteria, viruses, protozoa, or fungi—leads to lymphadenitis. Usually, an infection of the skin, ears, nose, or eyes, as well as illnesses like infectious mononucleosis, cytomegalovirus infection, streptococcal infection, tuberculosis, or syphilis, spread to a lymph node. The lymph nodes may be infected throughout the body or just in one particular region.

2.4.5 Small Skin Abscesses (pus-filled pockets in the skin)

Skin abscesses are pockets of infection beneath the skin's surface that are warm, painful, and filled with pus. They can develop on any part of the body. Abscesses can range in size from one to several inches. Skin abscesses include carbuncles and furuncles.

Furuncles: By definition, a hair follicle and the surrounding tissue are involved in furuncles (boils), which are sensitive, smaller, more superficial abscesses. On the neck, breasts, face, and buttocks, furuncles are typical. They hurt, especially in delicate regions, and are uncomfortable (for example, on the nose, ear, or fingers).

Carbuncles: Furuncles that are joined to one another underneath the skin's surface become carbuncles. Abscesses frequently come to a head and rupture if left untreated, releasing a creamy white or pink fluid. The lymph nodes and surrounding tissue may get infected by

bacteria that have migrated from the abscess. The person may feel generally ill and have a temperature.

Skin abscesses are identified by doctors based on how they appear. In order to identify the bacteria, doctors occasionally send pus samples to a laboratory (called a culture). Warm compresses may help an abscessed skin area heal. Otherwise, a doctor will cut open the abscess and drain the pus. A doctor drains the abscess, ensures that all of the pus is gone, and then cleans out the pocket with a sterile saline solution. More serious bacterial skin and skin structure infections include Cellulitis.

2.4.6 Cellulitis

Cellulitis is a bacterial infection of the skin and the tissues immediately below the skin (SSTI).

The bacteria *Streptococcus* and *Staphylococcus* are the most frequent causes of cellulitis. Since they create enzymes that prevent the tissue from containing the infection, *streptococci* spread quickly in the skin. Staphylococcal cellulitis typically develops around open wounds and pus-filled pockets (skin abscesses).

Cellulitis can be caused by a wide variety of bacteria. A form of *Staphylococcus* that was once susceptible to drugs is now a more frequent cause of cellulitis. Methicillin-resistant *Staphylococcus aureus* is the name of this strain (MRSA). A specific strain of MRSA that is frequently acquired in hospitals or nursing homes may react differently to antibiotics than other strains of MRSA that are more widespread outside of healthcare facilities.

Bacteria typically enter the body through tiny tears in the skin that are caused by animal bites, surgeries, burns, fungal infections, burns, and scrapes. Edematous (fluid-filled) cutaneous lesions are particularly susceptible. Cellulitis, however, can also develop in skin that is not visibly wounded.

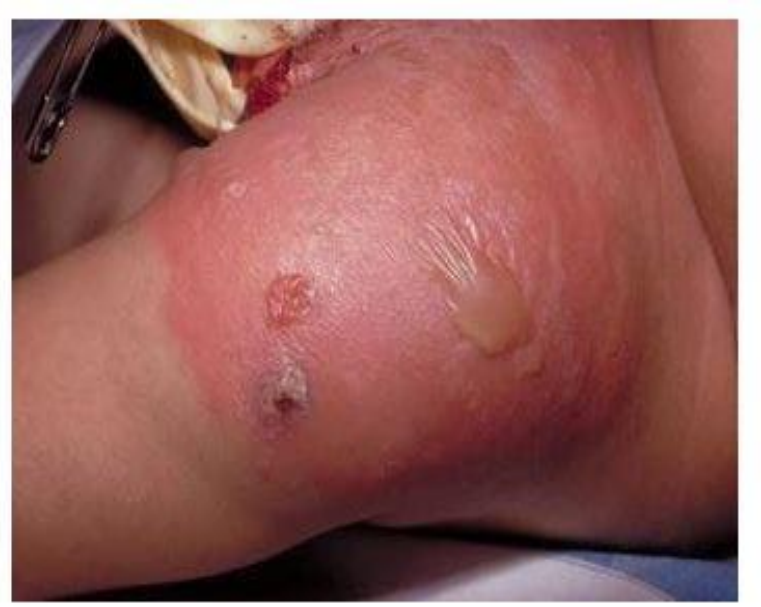

Plate 2.6: Red, Warm Areas on the Skin forming Cellulitis Related Blisters

Source[20]

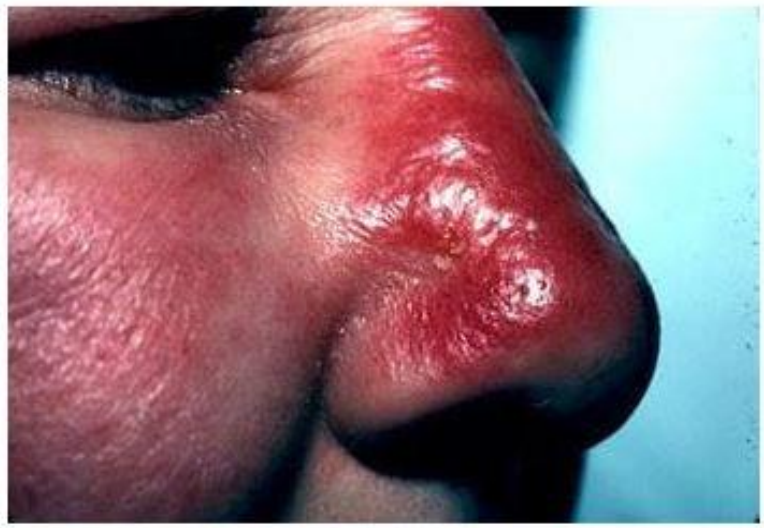

Plate 2.7: Erysipelas showing a red Skin with a raised edge and distinct border.

Source[20]

2.4.7 Erysipelas

Erysipelas is a superficial skin infection (cellulitis) most frequently caused on by *Streptococci*, though it can also be brought on by other bacteria like *Staphylococcus aureus*, including a strain known as methicillin-resistant *Staphylococcus aureus* (MRSA). Erysipelas typically affects the legs and face. A glossy, painful, red, elevated patch of skin is the result of erysipelas. The edges stand out from the surrounding normal skin and have defined borders. To the touch, the patch is firm and warm. The legs and face are where it happens most frequently. In some cases of erysipelas, skin blisters develop. Individuals frequently experience chills, a high fever, and other symptoms of disease (malaise).

The infection can be cured with oral antibiotics like penicillin. Trimethoprim/sulfamethoxazole, clindamycin, or doxycycline can be administered orally if doctors suspect a patient has MRSA; but, if the infection is severe, vancomycin or linezolid must be injected. Using cold packs and painkillers may make you feel better. In order to prevent recurrence, fungal foot infections may act as an infection entrance point and need to be treated with antifungal medications. Erysipelas-related leg edema can be reduced by compression stockings.

2.4.8 Lymphangitis

Lymphangitis is an infection of one or more lymphatic vessels, typically caused by *Streptococci*.

Streptococci bacteria can enter lymphatic vessels, typically through a scrape or wound on the arm or leg. Frequently, cellulitis, a streptococcal infection of the skin and tissues close below the epidermis, extends to the lymph vessels. *Staphylococci* or other bacteria are rarely responsible.

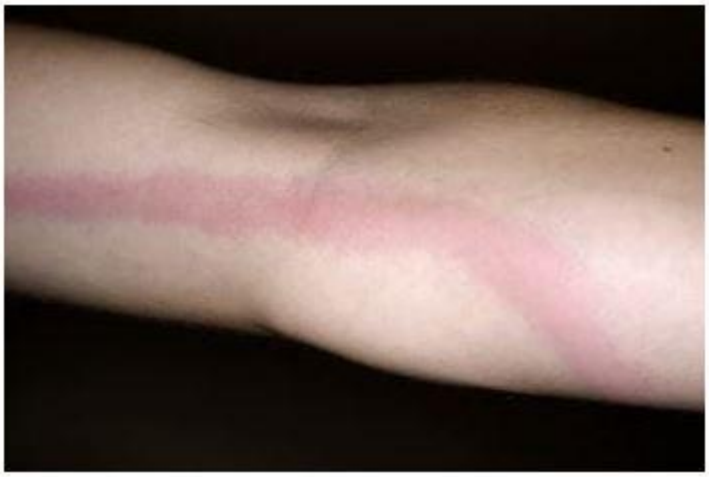

Plate 2.8: Patient experiencing a Crimson Track on their arm following an Insect Bite Consistent with Lymphangitis.

Source[20]

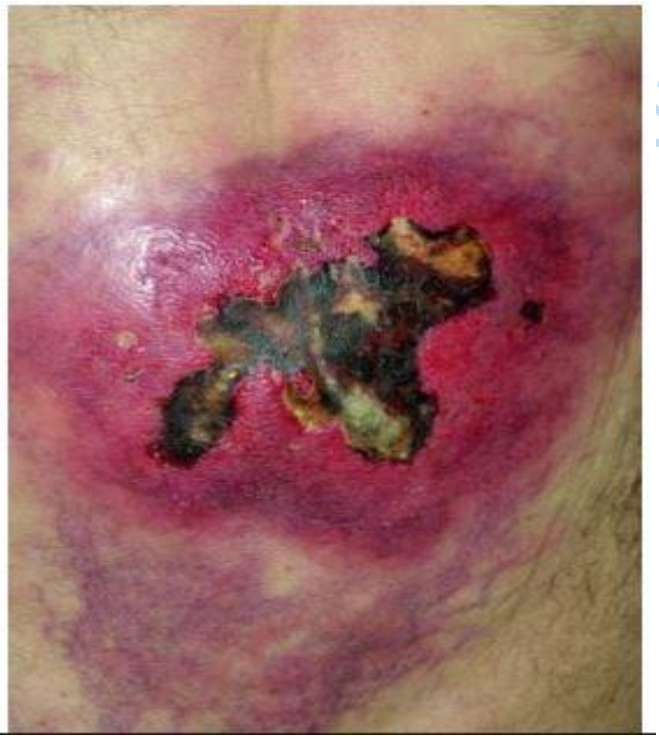

Plate 2.9: A Streptococcal Skin Infection on the Lower Back that is Potentially Fatal.

Source[20]

2.4.9 Necrotizing Skin Infections

The death of infected skin and tissues is a hallmark of necrotizing skin infections, which include necrotizing cellulitis and necrotizing fasciitis (necrosis).

The majority of skin infections do not kill the skin or the tissues around it. Small blood vessels in the affected area can clot occasionally as a result of bacterial infection. The tissue nourished by these veins dies from a lack of blood as a result of this clotting. Necrotic refers to dead tissue. The infection spreads quickly and may be challenging to control since the body's immunological defenses that travel through the bloodstream (such as white blood cells and antibodies) can no longer reach this location. Even with adequate treatment, death is still possible.

Certain necrotizing skin infections, also known as necrotizing fasciitis, spread deeply under the skin along the fascia, the connective tissue that covers muscles. Necrotizing cellulitis is the name given to other skin infections that cause necrosis and spread to the skin's outer layers. Necrotizing skin infections can be brought on by a variety of bacteria, including *Streptococcus* and *Clostridia*, but in many cases, many bacteria are to blame. The term "flesh-eating illness" has been used by the lay press to refer to a specific Streptococcal necrotizing cutaneous infection that is similar to the others.

Some specific skin infections that cause necrosis start at puncture wounds or lacerations, especially those that are covered with dirt and debris. Some infections start in wounds from surgery or even on healthy skin. Necrotizing infections of the abdomen wall, vaginal region, or thighs can occur in persons with diverticulitis, intestinal perforation, or intestine tumors. When specific bacteria escape from the colon and travel to the skin, infections like these can develop. At the beginning, the bacteria may form an abscess (a pocket of pus) in the abdominal cavity, from which they may then either travel externally to the skin or

internally through the bloodstream to other organs and skin. Diabetes patients are especially vulnerable to necrotizing skin infections.

2.4.10 Wound Infections

Staphylococcal scalded skin syndrome, scarlet fever, and toxic shock syndrome are skin-related consequences of bacterial infections. The skin can become infected by a variety of microorganisms. *Staphylococcus* and *Streptococcus* are the most prevalent. Methicillin-resistant *Staphylococcus aureus*, also known as MRSA, is a typical bacterium that causes skin infections. Because MRSA has undergone genetic alterations that enable it to live despite exposure to some antibiotics, it is resistant to several routinely used antibiotics. Doctors customize their treatment based on how frequently MRSA is found in the area and whether or not it has been confirmed to be resistant to regularly used medicines because MRSA is resistant to various drugs that were once used to kill it[20].

2.5 Theoretical Framework

One scientific theory that relates to the synergistic action between bacteriocin and antibiotics against MRSA and bacterial skin infections is the concept of antibiotic synergy. Antibiotic synergy refers to the phenomenon where the combined use of two or more antibiotics results in a greater antibacterial effect than would be expected from the individual drugs used alone[22].

Another theory that relates to this study is the concept of bacteriocins as a potential alternative to antibiotics for the treatment of bacterial infections. Bacteriocins are antimicrobial peptides produced by bacteria that can kill or inhibit the growth of other bacteria. Bacteriocins have been shown to have a broad spectrum of activity against many different bacterial species, including MRSA, and may be particularly useful in the treatment of antibiotic-resistant infections.

Furthermore, the theory of bacterial quorum sensing can also be relevant to this topic. Quorum sensing is a communication mechanism used by bacteria to coordinate their behavior, including the production of virulence factors and antibiotic resistance. By disrupting quorum sensing, it may be possible to enhance the efficacy of antibiotics and bacteriocins against MRSA and other bacterial pathogens.

Finally, the theory of antibiotic resistance evolution may also be relevant to this topic. Antibiotic resistance is a complex and rapidly evolving phenomenon, driven by the selective pressure of antibiotic use. By combining antibiotics with bacteriocins or other antimicrobial agents, it may be possible to slow the evolution of antibiotic resistance and prolong the useful lifespan of existing antibiotics.

2.6 Review of Empirical Studies

There have been numerous empirical studies conducted on the synergistic action between bacteriocin and antibiotics against MRSA and bacterial skin infections. MRSA is a major health concern due to its resistance to most antibiotics, and bacterial skin infections are common worldwide. Therefore, discovering novel ways to treat these infections is crucial[23].

One study published in the World journal of pharmaceutical research in 2018 investigated the synergistic effects of partially purified bacteriocin from *Cronobacter sakazakii* and various antibiotics against MRSA and various pathogenic bacteria. The study showed that the highest synergestic effect was obtained when bacteriocin was mixed with both amikacin and tetracycline in a ratio of 3:1, where the inhibition zones were increased to 29.4 mm and 27.3 mm respectively because the higher concentration of bacteriocins act as membrane lysis that lead to enhance the actions of antibiotic. The bacteriocin in higher concentration can potentiate the actions of antibiotic and reduce side effect of antibiotic and diminish resistant antibiotic strains.

Partial purification of bacteriocin from *Cronobacter sakazakii* was performed by ion exchange chromatography, then antibacterial activity of bacteriocin produced by *C. sakazakii* was studied by detecting the inhibitory effect of bacteriocin against *E. coli*, *S. aureus*, *K. penumoniae*, *S. dysenteriae*, *P. vulgaris* and *S. marcescens*, grown on BHI agar plates. The antagonistic effect against the test microorganism was done according to the well diffusion assay method.

The study concluded that bacteriocin-antibiotic combinations could be a viable treatment option for MRSA infections[24].

Another study published in the vaccines and molecular therapeutics section of the journal frontiers in immunology in 2021 investigated a synergy between the Thopeptide bacteriocin Micrococcin P1 and Rifampicin against MRSA in a murine skin infection model. The investigation showed that MP1 was very potent against MRSA but the inhibitory effect was overshadowed by resistance development during longer incubation time (24h or more). To overcome this problem a synergy study was performed with a number of commercially available antibiotics. Among the antibiotics tested, the combination of MP1 and rifampicin gave the best synergistic effect, with MIC values 25 and 60 times lower than for the individual drugs, respectively. To assess the therapeutic potential of the MP1-rifampicin combination, a murine skin infection model based on the use of the multidrug-resistant luciferase-tagged MRSA strain Xen31 was used. As expected, neither of the single antimicrobials (MP1 or rifampicin) could eradicate Xen31 from the wounds. By contrary, the results showed that MP1-rifampicin combination was efficient not only to eradicate but also to prevent the recurrence of Xen31 infection[25].

Another study published in the Journal of Biotechnology reports in 2016 investigated the synergistic effects of haloduracin a lantibiotic bacteriocin, and chloramphenicol against

clinically important Gram-positive bacteria. The study deals with evaluation of the potential synergistic_effect of haloduracin and chloramphenicol against Gram-positive pathogens with the aim of reducing the effective dose of chloramphenicol.

The combined use of haloduracin and chloramphenicol resulted in remarkable synergy against a spectrum of microorganisms including strains of *Staphylococcus aureus*, *Enterococcus faecium, Enterococcus faecalis* and different groups of *Streptococcus.* The synergy allowed the use of these antimicrobial agents at substantially reduced concentrations without compromising their efficiency. Use of lower doses of chloramphenicol can avoid the severity of its side effects. Chloramphenicol has a number of advantageous characteristics, including being a broad range antibiotic, diffusing effectively within the body, and not ionizing under physiological conditions. Yet its usage has been constrained due to the evolution of antibiotic resistance and its dose-related toxicity, which results in aplastic anemia, leukemia, bone marrow suppression, and gray baby syndrome. In addition to minimizing undesirable side effects of some drugs, this approach brings the possibility of using antibiotics that are no longer effective due to drug resistance. Furthermore, the observed synergy between haloduracin and chloramphenicol opens a new window of using bacteriocins and antibiotics in combination therapy of infections[26].

The alarmingly high rate of treatment resistance among diseases necessitates the creation of novel medications. New medication research is a time-consuming and expensive process, and in addition, microorganisms quickly acquire resistance to new medications. This may reduce the anticipated commercial life cycle (of the medications), making it less appealing for the major pharmaceutical corporations to invest in the discovery and development of new antibiotics. In summary, the empirical studies suggest that using antibiotics that are now available efficiently and effectively is crucial, and combination antibiotics have proved beneficial in this regard in boosting drug efficacy and reducing the emergence of drug

resistance[24]. Few studies have been focused on investigating the combined effect of non-antibiotic antibacterial agents and antibiotics against different pathogens. However, further research is needed to determine the optimal dosages and treatment durations of the bacteriocin-antibiotic combination for effective treatment.

2.7 Summary of Gap in Literature Reviewed

While some studies have explored the synergy between bacteriocins and antibiotics, there is a lack of comprehensive research on the optimal combinations of specific bacteriocins and antibiotics against resistant strains of bacteria associated with skin infections (e.g., MRSA, or *Pseudomonas aeruginosa*). Existing literature often focuses on the general concept of synergy, but the specific pairings and dosages required to combat drug-resistant pathogens such as MRSA, Multidrug-resistant K. pneumonia and Fungi remain largely uncharted[27].

The Significance of this is that it impedes the translation of theoretical synergy into practical therapeutic strategies. Identifying the most effective combinations crucial for developing targeted and efficient treatments for skin infections caused by Multidrug-resistant bacteria.

Another notable omission is a need for in-depth investigations into the mechanisms by which bacteriocins and antibiotics interact synergistically against drug-resistant skin pathogens, such as elucidating whether this synergy involves inhibition of resistance mechanisms, biofilm disruption, or other mechanisms at play. While studies have hinted at the synergy's existence, the precise mechanisms involved remain unclear. Understanding whether this synergy operates through the inhibition of resistance mechanisms, biofilm disruption, or other unidentified mechanisms is essential for developing a mechanistic framework to guide therapeutic development[28].

This is crucial because the lack of mechanistic understanding hinders the rational design of combination therapies. Elucidating these mechanisms is vital for fine-tuning treatment strategies and potentially uncovering novel targets for intervention.

While in vitro studies have shown promise, there is a limited understanding of the in vivo efficacy and safety of combining bacteriocins and antibiotics for the treatment of skin infections. Most research to date has focused on laboratory settings, and there is a scarcity of data regarding the practicality and potential side effects of this combination therapy when applied in living organisms[29].

The transition from in vitro studies to in vivo models and clinical settings is critical for assessing the translational potential of bacteriocin-antibiotic synergy. Robust in vivo data are essential for evaluating safety, effectiveness, and potential challenges associated with this treatment approach.

Furthermore, the impact of using bacteriocins and antibiotics in combination on the normal skin microbiota remains poorly explored. Understanding how this combination therapy affects the beneficial skin microbiota is crucial for assessing its clinical applicability, especially regarding the potential for unintended consequences such as dysbiosis[30].

This is significant because the skin microbiota plays a vital role in maintaining skin health and preventing pathogenic colonization. Neglecting its impact may lead to unintended consequences, including prolonged skin issues or increased vulnerability to infections.

Research on the potential for the development of resistance to bacteriocins and antibiotics in the presence of each other is currently scarce. Most studies tend to focus on resistance to individual agents, but there is a pressing need to investigate the evolution of resistance mechanisms under combination therapy. Understanding how bacteria may adapt and develop resistance in response to bacteriocins and antibiotics used together is vital for ensuring the

long-term effectiveness of this treatment approach[31]. If resistance to combination therapy arises, it could undermine the efficacy of this promising treatment option. Research in this area is essential to anticipate and mitigate the development of resistance

Also, despite the potential benefits of bacteriocin-antibiotic combination therapy, there is a lack of comprehensive cost-effectiveness analyses comparing it to traditional treatment modalities for skin infections. Factors such as treatment duration, hospitalization costs, recurrence rates, and the economic burden on healthcare systems have not been adequately addressed in the literature. A thorough cost-effectiveness analysis is crucial for determining the economic viability and sustainability of this treatment approach in real-world healthcare settings[32].

This is significant because cost-effectiveness analysis is vital for informing healthcare policy decisions and resource allocation. Without this information, it is challenging to assess whether the benefits of bacteriocin-antibiotic combination therapy outweigh the costs and resource requirements.

Little attention has also been given to the growing body of research on the antimicrobial properties of bacteriocins and their synergistic effects with antibiotics, there is a notable gap in our understanding of how bacteriocins modulate the host immune response during skin infections caused by drug-resistant bacteria. The current literature tends to focus on the direct antibacterial activity of these compounds, but their potential immunomodulatory effects have not been comprehensively explored[33]. Understanding the role of bacteriocins in modulating the host immune response is crucial for several reasons:

i. Enhanced Immune Activation: Bacteriocins may have the capacity to enhance the host's immune response against bacterial infections. Investigating their impact on

immune cells, such as macrophages, neutrophils, and dendritic cells, could reveal novel mechanisms for bolstering the host's defenses against resistant bacterial strains.

ii. Inflammatory Responses: There is a need to assess whether bacteriocins contribute to the regulation of pro-inflammatory and anti-inflammatory responses in the skin. This knowledge is vital for designing therapies that not only eliminate bacteria but also mitigate excessive inflammation, which is often associated with skin infections.

iii. Immunomodulation in Immunocompromised Patients: For patients with compromised immune systems, such as those with diabetes or immunosuppressive conditions, understanding how bacteriocins affect the immune response becomes even more critical. Are there risks of overstimulation or suppression of immune function?.

Lastly, while the use of bacteriocins and antibiotics in combination shows promise in combating drug-resistant bacteria, there is a significant gap in our understanding of whether this approach might inadvertently lead to the emergence of new bacterial strains with increased virulence. The potential for bacteriocins and antibiotics to exert selective pressure on bacteria, favoring the survival and proliferation of more virulent strains, remains largely unexplored[14].

This gap is critical due to the following reasons:

i. Risk of Resistance Evolution: The potential for the development of resistance to bacteriocins and antibiotics has been widely discussed, but the accompanying risk of bacterial strains evolving to become more virulent in response to these compounds has received less attention.

ii. Clinical Implications: If more virulent bacterial strains emerge as a consequence of combination therapy, it could have severe clinical implications, including treatment failures, increased disease severity, and heightened transmission potential.

iii. Understanding the Evolutionary Dynamics: Investigating the genetic and phenotypic changes that bacteria may undergo in the presence of bacteriocins and antibiotics is crucial for a comprehensive assessment of the risks associated with this treatment approach.

iv. Mitigation Strategies: Identifying strategies to mitigate the emergence of more virulent strains, such as dose optimization or combination therapy protocols, is essential for ensuring the long-term effectiveness of bacteriocin-antibiotic therapy.

Addressing these gaps in the literature will contribute to a more comprehensive understanding of the potential benefits and risks associated with using bacteriocins and antibiotics as synergistic therapeutic agents against drug-resistant bacteria in the context of skin infections.

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Chapter Three

Methodology

3.1 Research Design

This research work was conducted in two stages, and it is directed to identify the effect of two different antimicrobials: Bacteriocins produced by Lactic Acid Bacteria and Antibiotics (Penicllin). The first stage involved location profile determination, and sample collection for both milk samples and Test microorganism samples. The analytical stage, which took place in the second stage, entails sample preparation, laboratory bench work, and instrument analysis.

3.2 Study Area

The University College hospital (UCH) with coordinate's 7.357133°N 3.874262°E is tactically located in Ibadan, the then largest city in West Africa. The structural development of the Hospital commenced in 1953 in its present site and was formally commissioned after completion on 20th of November 1957. The University College Hospital, Ibadan was initially commissioned with 500-bed spaces. Currently, the hospital has over 1500 bed spaces and 163 examination couches with occupancy rates ranging from 55-60%[1].

Ido Local Government Area with coordinate's 7°30'24.41" N 3°42'42.70" E is the largest local government area in Ibadan, Oyo State. It serves as the city's commercial and industrial district. It extends from the Orita Challenge, Apata, and Eleyele Ologuneru axis towards

Eruwa. Its corridors are known for developing quickly. It has a 986 km2 area and 103,261 people as of the 2006 census. It shares boundaries with Oluyole, with coordinate's 7.218°N 3.892°E which is one of the oldest Local government council in Oyo state[3]. Within Ibadan Metropolis, it shares borders with four LGAs: Ibadan South-West, Ibadan South-East, Ona-Ara, and Ido. Through the local governments of Egbeda-Obafemi, Odeda, and Ijebu-North, it borders Ogun State.

The Council of Oluyole Local Government was founded in 1976 and covers a territory of around 4,000 square kilometres with a population of 202,725 as of the 2006 Census[4].

Fig 3.1: A cross-section of University College Hospital, Ibadan.

Source[2]

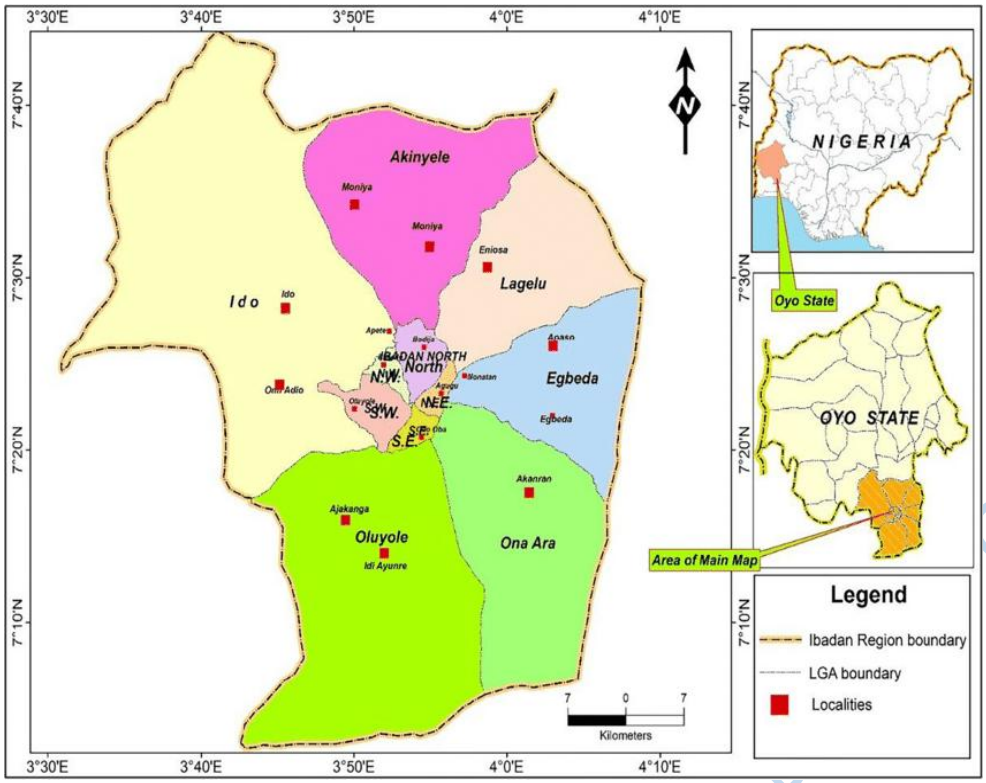

Fig 3.2: Map of Ibadan Showing Ido and Oluyole Local Governments Areas of Ibadan, Oyo state with Coordinates

Source[5]

3.3 Sample Collection

3.3.1 Collection and Preparation of Test Microorganisms

All pathogenic strains used in this study were isolated from Skin ulcers and infections from patients at the University Teaching Hospital (UCH), Ibadan, Oyo State. They were kept in Tryptic soy broth and these strains include: *Acinetobacter baumanii, Salmonella sp,* Methicillin Resistant *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Enterobacter cloacae, Enterobacter aerogenes, Pseudomonas aeruginosa, Raoultella ornithinolytica, Serratia odorifera,* Multi-drug resistant *Klebsiella pneumoniae, Candida sp, Aeromonas hydrophilia.* All strains were grown aerobically for 18h at 37°C on Nutrient agar to confirm viability of organisms and were also subjected to Gram staining and

oxidase test to re-identify the isolates as members of the family *Enterobacteriaceae* and members of the family *Pseudomonadaceae*.

3.3.2 Collection of Fresh Cow Milk

Samples of fresh cow milk samples were randomly collected from lactating cows in Oluyole and Ido local government areas of Ibadan, Oyo State, Nigeria. The raw milk samples were collected into sterile sample bottles and transported immediately to the Lead City University Microbiology Laboratory for bacteriological analysis.

3.4 Isolation and Characterization of Lactic Acid Bacteria from Fermented Milk

Bacteriocin producing LAB isolation was performed using serial dilution. The MRS agar to be used is first prepared by suspending 17.05g of MRS agar in 250ml of distilled water and autoclaved for 15minutes at 121°C, allowed to cool and dispensed into sterile petri-dishes. Spread plate method was used by spreading 0.1ml of fresh cow milk on de Mann Rogosa and Sharpe agar (MRS) with the use of a spreader. MRS agar was previously sterilized at 121°C for 15minutes, left to cool and supplemented with 2g of Fungusol antifungal powder for 1000ml of MRS agar to prevent the growth of yeast (as yeast and fungi very easily grow on MRS agar) before pouring into sterile petri-dishes. The inoculated plates were incubated at 37°C for 48 hours with the use of an anaerobic jar to achieve anaerobic condition. Isolated colonies with typical characteristics of LAB were then purified by streaking them over the same agar medium aseptically and incubated anaerobically at the same temperature for 48 hours to obtain pure cultures. Each pure colonies obtained was then subjected to Gram staining and biochemical tests[6]. The results were used to identify the isolates and obtained[7].

The molecular identification of the isolate was achieved by 16rRNA gene sequencing carried out by the Clinical virology molecular laboratory of University of Ibadan Teaching Hospital, Ibadan, Oyo State.

3.5 Molecular Identification of Isolates.

3.5.1 DNA Extraction

Briefly, Single colonies grown on medium were transferred to 1.5 ml of liquid medium and cultures were grown on a shaker for 48 h at 28 ºC. After this period, cultures were centrifuged at 4600g for 5 min. The resulting pellets were resuspended in 520 µl of TE buffer (10 mMTris-HCl, 1mM EDTA, pH 8.0). Fifteen microliters of 20% SDS and 3 µl of Proteinase K (20 mg/ml) were then added. The mixture was incubated for 1 hour at 37 ºC, then 100 µl of 5 M NaCl and 80 µL of a 10% CTAB solution in 0.7 M NaCl were added and votexed. The suspension was incubated for 10 min at 65 ºC and kept on ice for 15 min. An equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by incubation on ice for 5 min and centrifugation at 7200g for 20 min. The aqueous phase was then transferred to a new tube and isopropanol (1: 0.6) was added and DNA precipitated at –20 ºC for 16 h. DNA was collected by centrifugation at 13000g for 10 min, washed with 500 µl of 70% ethanol, air-dried at room temperature for approximately three hours and finally dissolved in 50 µl of TE buffer.

3.5.2 Polymerase Chain Reaction

PCR sequencing preparation cocktail consisted of 10 µl of 5x GoTaq colourless reaction, 3 µl of 25mM MgCl2, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol each 27F 5'- AGA GTT TGA TCM TGG CTC AG-3' and - 1525R, 5′-AAGGAGGTGATCCAGCC-3′ primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water 8µl DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a PCR profile consisting of an initial denaturation at 94°C for 5 min; followed by a 30 cycles consisting of 94°C for 30 s, 50°C for 60s and 72°C for 1 minute 30 seconds ; and a final termination at 72°C for 10 mins. And chill at 4oC.GEL (2,3).

3.5.3 Integrity

The integrity of the amplified 1.5Mb gene fragment was checked on a 1% Agarose gel ran to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2 l) of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4µl of each PCR product and loaded into the wells after the 100bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel[6].

3.5.4 Purification of Amplified Product

After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6 µl of Na acetate 3M and 240 µl of 95% ethanol were added to each about 40µl PCR amplified product in a new sterile 1.5 µl tube eppendorf, mix thoroughly by vortexing and keep at -20°C for at least 30 min. Centrifugation for 10 min at 13000 g and 4°C followed by removal of supernatant (invert tube on trash once) after which the pellet were washed by adding 150 µl of 70% ethanol and mix then centrifuge for 15 min at 7500 g and 4°C. Again remove all supernatant (invert tube on trash) and invert tube on paper tissue and

let it dry in the fume hood at room temperature for 10-15 min. then resuspend with 20 µl of sterile distilled water and kept in -20oC prior to sequencing. The purified fragment was checked on a 1.5% Agarose gel ran on a voltage of 110V for about 1hr as previous, to confirm the presence of the purified product and quantified using a nanodrop of model 2000 from thermo scientific[6].

3.5.5 Sequencing

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA 6 were used for all genetic analysis[6].

3.6 Biochemical Characteristics Tests

All 3 LAB isolates coded as NG1, NG2, ELY were subjected to the following biochemical tests: Gram staining, IMViC (Indole, Methyl red, Voges- Proskauer and Citrate), Motility, and Sugar fermentation.

3.6.1 Gram Staining

Thin smear of about 200mm in diameter was made on grease free slide and fixed over a burning flame by passing the slide over the flame about 2-3 times. A 0.5% crystal violet solution was applied to cover the smear for 20 seconds and rinsed with distilled water. Next, Gram's iodine was applied to the surface for 30 seconds and equally rinsed out. Acetone was then used to decolorize the stain and rinsed immediately over a fast stream of running water before counterstaining using safranin solution for 1 minute, rinsed and allowed to dry. Each isolate was blot dried and observed under the microscope with oil immersion for pink or purple stains[6].

3.6.2 Indole Test: A wire loop was used to inoculate overnight growth cultures into a test tube of 5ml peptone water. The inoculation was incubated at 37°C for 24 hours after which 5 drops of Kovac's indole reagent was added and shaken gently. A positive reaction was indicated by the development of a red color formation on the top layer[6].

3.6.3 Methyl Red- Voges-Proskauer: Organism was grown in 5ml MR-VP broth and incubated for 48-72 hrs at 35°C after incubation, 1ml of the broth was transferred into a test tube and 2-3 drops of methyl red was added. Formation of red color indicates positive methyl red test, a yellow color indicates negative test. To the rest of the broth, 15 drops of 15% alpha – napthal in alcohol was added. 5 drops of 40% KOH was also added followed with shaken gently. The cap of the tube was loosened and development of a red color within 1hr indicates a positive test. No color change indicates negative test[6].

3.6.4 Citrate Test: The isolates were inoculated on 2.428g of Simmon's citrate agar suspended in 100ml of distilled water and sterilized at 121°c for 15 minutes in petri-dishes and incubated for 24- 72hrs. Development of a deep blue colour indicates a positive reaction[6].

3.6.5 Motility: The isolates were inoculated into the motility medium by making a fine stab with a needle to a depth of 1-2cm long in the tube. It was then incubated at 35°c for 24 48hrs. Sharply undefined line of inoculation and cloudiness in the media indicates a positive result (organism is motile). Sharply defined and restricted growth to the line of inoculation indicates a negative result[6].

3.6.6 Catalase Test: LAB isolates were grown in MRS broth for 18-24 hours prior and 1-2 ml of hydrogen peroxide solution were poured into three test tubes. Using a syringe or pipette, 2-3 drops of the isolates were immersed in the hydrogen peroxide solution and observed for immediate bubbling. No signs of bubbling indicates a catalase negative result[7].

3.6.7 Sugar fermentation Test: Sorbitol, mannitol, fructose, glucose and lactose sugars were used for this sugar fermentation test. The selected carbohydrate mentioned above were

dissolved in 1000ml distilled water mixed with 0.018g of Phenol red (indicator) and sterilized at 121°c for 15 minutes. 5-7ml of the mixture was then dispensed into bottles with Durham tubes submerged completely into each bottle and observed for the absence of air bubbles. Each of the bottles were then inoculated with the LAB colonies from a pure broth culture of the organism, with one bottle left out serving as control. Bottles were then incubate anaerobically at 35-37ºC for 3-5 days[7].

3.7 Production of Bacteriocin

For bacteriocin production, each LAB isolate was inoculated in 250ml of MRS broth (adjusted to a pH 6.5), dispensed into 250ml Erlenmeyer flask and incubated in an anaerobic jar and maintained at 37°C for 48 hours. After incubation, cells were removed from the growth medium by centrifugation at 5000rpm/min for 10 minutes. The cell free supernatant was collected and adjusted to pH 6.5 using a pH meter, and the activity was measured by standard well diffusion assay.

The cell free supernatant was divided into 2 equal sections: One section was used as crude bacteriocin and the second section was used in combination with Penicillin to form a synergistic mixture.

3.8 Antibiotic Susceptibility Test of Test Organisms

Antibiotic susceptibility of test organisms were done using Gram negative isolates and Gram positive isolates antibiotics disc by Celtech diagnostics. Gram negative antibiotics disc used include: (30µg Amoxicillin clavulanate, 30µg Penicillin, 5µg Oflaxacin, 25µg Cefotaxime, 10µg Gentamycin, 10µg Imipenem, 30µg Nalidicic Acid, 300µg Nitrofurantoin, 30µg Cefuroxime, 45µg Ceftriaxone sulbactam, 10µg Ampliclox, 5µg Cefexime, and 5µg Levofloxacin. Gram positive antibiotics disc used include: 15µg Erythromycin, 10µg

Gentamycin, 15µg Azithromycin, 10µg Imipenem, 30µg Amoxicillin clavulanate, 25µg Cefotaxime, 45µg Ceftriaxone sulbactam, 5µg Cefexime, 5µg Levofloxacin and 5µg Ciprofloxacin, 5µg Oxacillin, 30µ Chloramphenicol, 300µg Nitrofurantoin, and 5µ Rifampicin. The Mueller-Hinton agar was sterilized at 121°C for 15 minutes, allowed to cool, and then dispensed into sterile Petri dishes. Using a flame-sterilized inoculating loop, 0.5 Mcfarland standard was used for the test organisms to ensure consistent inoculum density and evenly spread onto the Mueller-Hinton agar plates. Subsequently, a Gram-negative antibiotic disc was firmly affixed in the center of each prepared Mueller-Hinton agar plate. The plates were then incubated overnight at 37˚C. After incubation, the diameter of the inhibition zone around each antibiotic disc was carefully measured using a transparent ruler and recorded.

3.9 Antibacterial Activity of Lactic Acid Bacteria on Pathogenic Bacteria

3.9.1 Agar-Well Diffusion Method

The antimicrobial activity of the cell free supernatant (CFS) produced by each LAB isolates were studied by detecting the inhibitory effect of the crude extract of bacterial broth assumed to contain bacteriocin against pathogenic isolates on Nutrient agar plates. The antagonistic effect against the test microorganism was done according to the well diffusion assay method[9]. 0.5 Mcfarland standard overnight culture of test microorganisms were taken and spread on Mueller-Hinton agar plates using inoculating loop. Immediately wells were made on the surface of the medium using sterile cork borer and filled with 75µl of crude filtrate of LAB isolates coded as NG1, NG2, and ELY, and 75µl of CFS and 2g of PenG. Following 24h incubation at 37°C (in the presence of atmospheric oxygen), ZDI (Zone Diameter of Inhibition) values (nearest whole) were recorded and interpreted as less active, moderately active, and highly active with ZDIs of 10mm, 11-14mm, and >15mm, respectively, around CFS extract[8]. The tests were run three times, and the findings were shown as ± (Standard

Deviation). The test *Lactobacilli*'s antagonistic actions were measured in arbitrary units per milliliter (AU/mL) using the following formula: AU/mL = $\frac{ZDI \times 1000}{Volume\ taken\ in\ the\ well\ (\mu l)}$ where ZDI stands for "Zone Diameter of Inhibition[10].

3.9.2 Agar Overlay Method

A loopful quivalent to (10^5 CFU/spot) of MRS broth culture that had been grown with each individual LAB isolate for 48 hours at 35°C were inoculated on the MRS agar plates. The inoculated plates were then incubated at 37°C for 48 hours and labeled appropriately. Following the solidification of the overlay agar medium, the agar plates containing the culture of LAB in spot form were overlaid with soft Mueller-Hinton agar (mixed with 0.8% bacteriological agar) and incubated at 37°C for 24 hours. The measured and interpreted zone diameter of inhibition (ZDI) values were ZDI >20mm, 10-20mm, and 10mm, which were regarded as strong intermediate and weak inhibitions, respectively. The diameter of the clear zone around the d spot and the diameter of the spot form of LAB grown on the MRS agar plate, respectively, were used to calculate the width of the clear zone values, using the formula

R= $\frac{(d\ Inhib - d\ spot)}{2}$ where (*d* Inhib: the diameter of clear zone around the *d* spot; and *d* spot: the diameter of spot form of LAB grown on MRS agar plate). The test isolates' growth inhibition scores were considered as low inhibition capacity when R was >2 mm, low inhibition capacity when R was between 2-5 mm, and high inhibition capacity when R was >6 mm. The data was represented as mean SD (Standard Deviation) and all tests were conducted in triplicates[10].

3.10 Synergistic Effect of Lactic Acid Bacteria and Antibiotics against Test Organisms

In order to detect the synergetic effect of CFS of Lactic acid bacteria with Penicillin, 250ml of Mueller Hinton agar was prepared and sterilized at 121°c for 15 minutes and left to cool. The agar was then dispensed into sterile petri-dishes, left to solidify and streaked with test organisms. A sterile borer was used to bore a hole into the agar and filled with already prepared synergestic mixture of the two antimicrobials. The synergestic mixture was prepared by combining 75µl of crude extract of LAB (grown anaerobically for 4 days to accumulate bacteriocin) with 2g of Penicillin injection bp in the ration 2:1 (2 being the LAB extract and 1 being Penicillin) and dispensed into the agar wells and incubated at 37°C for 24 hours and 48 hours respectively. The synergistic effect was determined by measuring the zone diameter of inhibition.

3.11 Method of Data Analysis

Three independent experiments were performed and all data were analysed by Standard Deviation and one-way ANOVA using Statistical Package for Social Science (SPSS) version 22. Differences among the means were tested for significance ($P<0.05$) by Duncan's post hoc test[11].

Endnotes

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Chapter Four

Results and Discussion of Findings

4.1 Results

A comprehensive overview of the results obtained from our study is presented below, showing the identification of Lactic Acid Bacteria used in this study, and the relationship between bacteria, fungi and antimicrobials (LAB and Antibiotics). Our presentation of results is organized into several sections, each structured to illuminate different facets of our research objectives. These findings shows our approach to the treatment of skin infections, offering potential breakthroughs in the battle against drug-resistant bacteria.

4.1.1 Results of Identification of Isolated LAB

The result of the biochemical tests and sugar fermentation tests of LAB coded as ELY, NG1 NG2 are presented below (Table 4.1& 4.2), following biochemical and morphological tests. All of these isolates revealed features of Gram-positive, non-spore-forming, non-motile, rod-shaped bacteria, except one NG2 (which showed features of Gram positive *cocci*). Furthermore, they tested negative for catalase, vogues-proskauer, and citrate tests, validating their categorization as members of the *Lactobacillus* genus. Identification of these isolates as *Lactobacillus* as well as the phylogenetic tree of the identified LAB were further validated using molecular identification and results recorded as reported in Table 4.3 and Fig 4.1.

The sequences of 16S rRNA gene products of the three LAB strains were estimated by comparison with the mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the agarose gel and gave 99.51% similarity to *Weissella paramesenteroides*, 99.93% similarity to *Limosilactobacillus fermentum* and 99.34% similarity to *Leuconostoc mesenteroides* respectively, as reported in Table 4.3.

Table 4.1: Biochemical Characteristics of Isolated *Lactobacillus spp*

	Lactobacillus isolates		
Biochemical Tests	NG1	NG2	ELY
Cell Shape	Rod	Cocci	Rod
Gram staining	+	+	+
Motility	–	–	–
Catalase Test	–	–	–
Citrate Test	–	–	–
Methyl-Red Test	–	+	+
Vogues-Proskauer Test	–	–	–

Table 4.2: Sugar Fermentation Tests of Isolated *Lactobacillus sp*

	Lactobacillus isolates		
Sugar Fermentation Tests	NG1	NG2	ELY
Glucose	+	+	+
Lactose	+	+	+
Sorbitol	+	+	+
Mannitol	+	+	+
Fructose	+	+	+

Table 4.3: Identification of LAB strains through 16S rRNA gene analysis

Lactobacilllus Sample code	Scientific Name	Max Score	Total Score	Query Cover	E value	Percentage Identity
NG1	*Weissella paramesenteroides*	2621	2621	100%	0	99.51%
NG2	*Limosilactobacillus fermentum*	2645	2645	99%	0	99.93%
ELY	*Leuconostoc mesenteroides*	2601	2601	97%	0	99.34%

NCBI Blast showing the sequence identity of the selected bacteria isolates

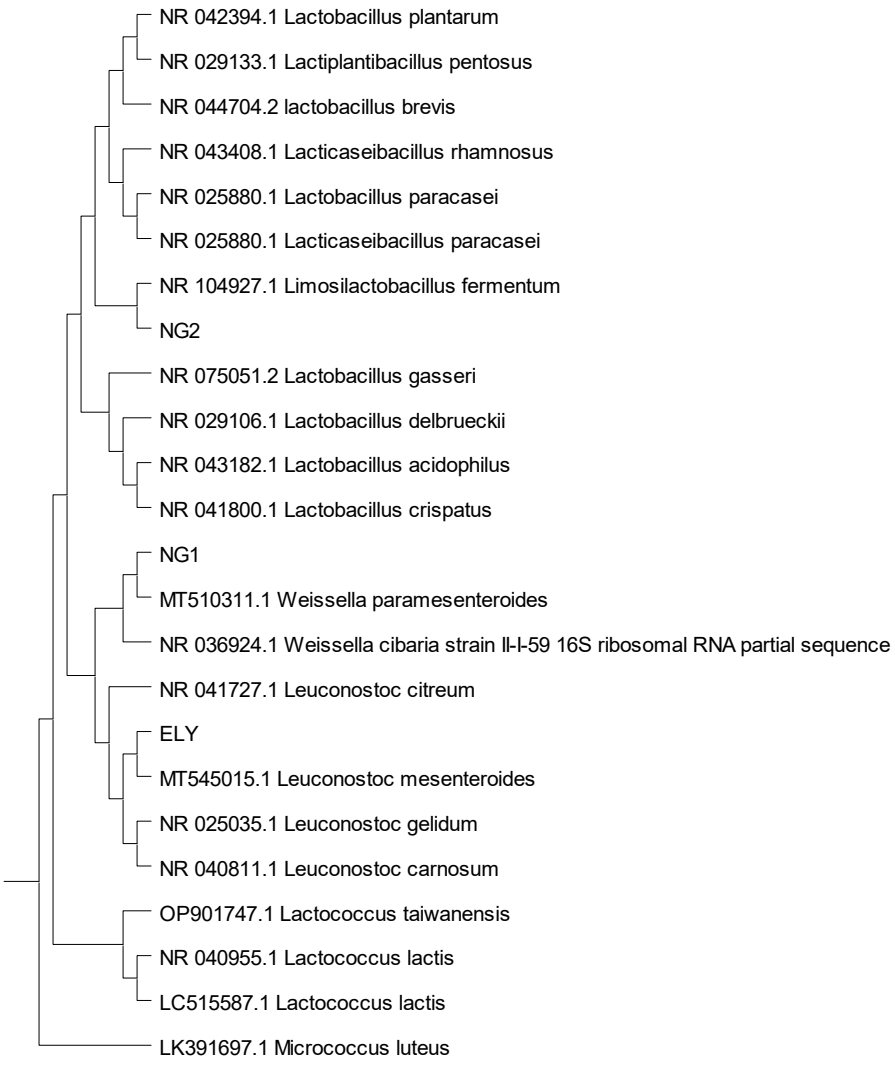


Fig 4.1: Phylogenetic tree of *Lactobacillus* isolates coded as (NG1, NG2, ELY)

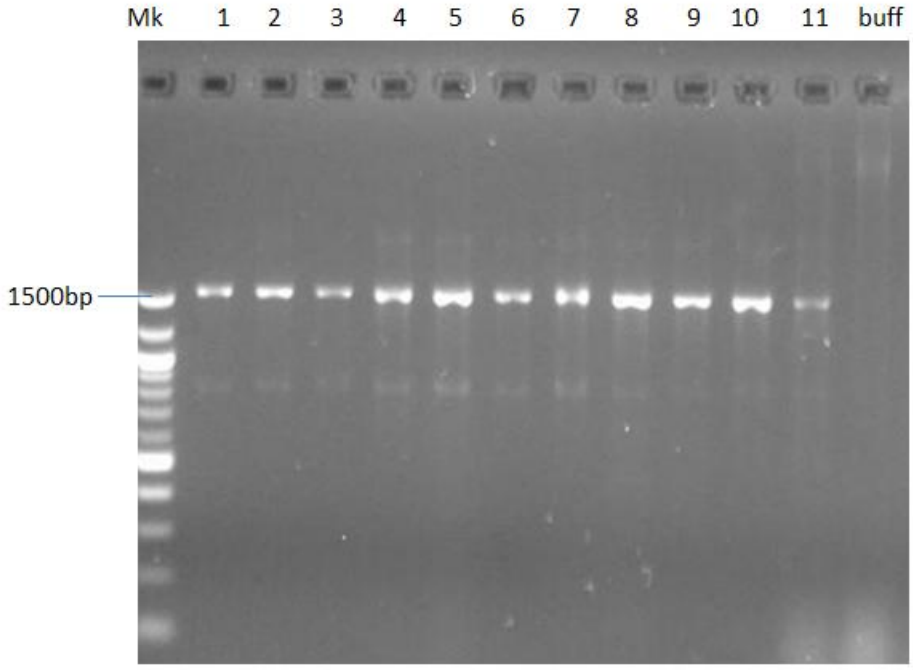


Fig 4.2: Agarose gels showing the positive amplification of the 16S rRNA partial gene amplified from the selected bacteria isolates.

4.1.2 Results of the Antibacterial Activity of LAB Strains

The "R" values, zone diameter of inhibition in standard deviation and AU/ml of the isolated LAB against the Gram-negative pathogenic bacteria following agar well diffusion and agar overlay methods are represented in Tables 4.4, 4.5, 4.6 and 4.7. The results of the synergistic activity of the combination of LAB isolates and Penicillin is also presented.

Table 4.4: The "R" Values of LAB Isolates against the Pathogenic Bacterial Isolates

Pathogenic Isolates	R Value (mm), Mean ± SD, for the Pathogenic Indicator Bacteria		
	NG1	NG2	ELY
MRSA	6.83±1.61	6.17±1.26	5.33±2.25
A.hydrophilia	0.50±0.50	-0.67±0.58	-2.00±0.87
A.baumanii	0.83±0.76	-1.00±1.32	-1.67±1.44
Candida	7.00±1.00	5.00±1.00	7.00±1.73
Salmonella	6.33±1.26	4.83±1.61	2.67±0.76
E.coli	2.50±1.00	3.17±1.26	1.33±1.26
E.cloacae	-1.00±1.32	-0.83±1.53	1.50±1.50
E. aerogenes	1.83±0.76	-1.83±1.15	5.17±2.25
Pseudomonas	-1.17±1.26	-2.00±0.87	4.67±2.75
K.pneumoniae	7.00±1.00	4.67±1.53	5.50±2.29
MDR K.pneumoniae	4.83±1.26	4.17±1.53	5.50±2.00
R.ornithinolytica	7.33±0.76	2.67±1.26	5.17±2.57
S.odorifera	2.33±0.58	3.00±1.80	1.17±1.04
P.mirabilis	0.00±1.00	-1.83±1.15	-2.17±0.58

"R": Zone of Clearance; SD: Standard Deviation.

Table 4.5: Antibacterial Activity of the Isolated LAB, in terms of ZDI, following Agar Overlay Method.

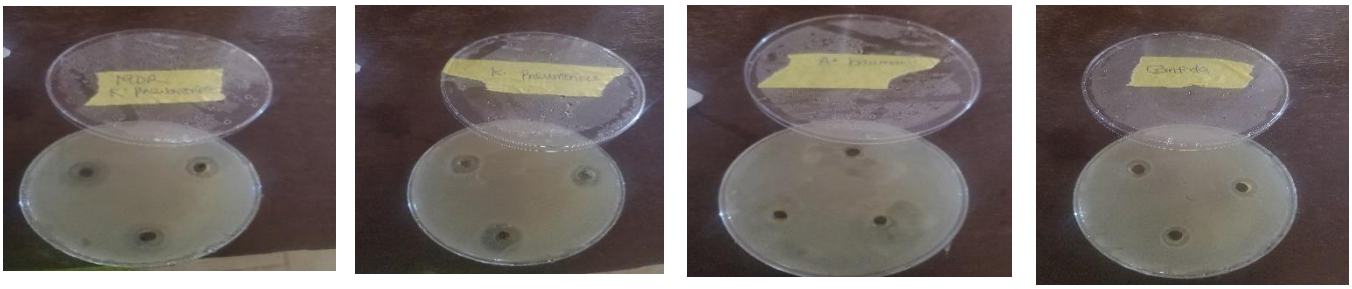
Pathogenic Isolates	ZDI (mm) ± SD for Indicator Bacteria		
	NG1	NG2	ELY
MRSA	18.67±3.21	17.33±2.52	15.67±4.51
A. hydrophilia	6.00±1.00	3.67±1.15	1.00±1.73
A. baumanii	6.67±1.53	3.00±2.65	1.67±2.89
Candida	19.00±2.00	15.00±2.00	19.00±3.46
Salmonella	17.67±2.52	14.67±3.21	10.33±1.53
E.coli	10.00±2.00	11.33±2.52	7.67±2.52
E. cloacae	3.00±2.65	3.33±3.06	8.00±3.00
E. aerogenes	8.67±1.53	1.33±2.31	15.33±4.51
Pseudomonas	2.67±2.52	1.00±1.73	14.33±5.51
K. pneumoniae	19.00±2.00	14.33±3.06	16.00±4.58
MDR K. pneumoniae	14.67±2.52	13.33±3.06	16.00±4.00
R. ornithinolytica	19.67±1.53	10.33±2.52	15.33±5.13
S. odorifera	9.67±1.15	11.00±3.61	7.33±2.08
P. mirabilis	5.00±2.00	1.33±2.31	0.67±1.15

ZDI: Zone Diameter of Inhibition; SD: Standard Deviation.

Table 4.6: Antibacterial Activity of LAB Isolates against the Indicator Bacterial Isolates following Agar-Well Diffusion Method

Pathogenic isolates	ZDI (mm) ± SD for Indicator Bacteria		
	ELY	NG1	NG2
MRSA	8.33±8.02	10.67±4.04	7.67±7.09
A. hydrophilia	0.13±0.23	2.00±2.00	2.00±2.00
A. baumanii	0.03±0.06	1.33±1.53	2.00±2.00
Candida	7.67±3.06	7.33±2.52	8.33±2.52
Salmonella	7.33±3.51	9.00±2.00	9.67±2.52
E.coli	10.67±4.51	7.00±2.00	20.00±9.00
E. cloacae	0.03±0.06	-	12±13.75
E. aerogenes	0.10±0.10	-	3.67±3.51
Pseudomonas	2.67±2.52	-	3.00±3.61
K. pneumoniae	11.33±5.13	8.50±3.28	11.00±2.65
MDR K. pneumoniae	5.33±5.51	7.50±3.77	9.00±4.00
R. ornithinolytica	6.33±7.09	2.67±2.52	9.00±4.58
S. odorifera	2.00±2.00	0.70±1.13	1.00±1.73
P. mirabilis	3.00±2.65	-	0.67±1.15

ZDI: Zone Diameter of Inhibition; SD: Standard Deviation.



MDR *K. pneumoniae* *K. pneumoniae* *A. baumanii* *Candida*

Bacterial pathogens

Plate 4.1: Agar-well diffusion technique demonstrates the antibacterial activity of LAB isolates against fourteen bacterial isolates used as indicator strain. The Zones of Inhibition is seen around the three LAB strains (injected into each hole) against three of the four bacterial pathogens shown above (*MDR K. pneumoniae, K. pneumoniae*, and *Candida*) (*A. baumanii* showed little to no inhibition).

Table 4.7: Growth Inhibitory Activity of LAB expressed in "AU/mL" for Bacterial Pathogens.

Pathogenic Bacteria	Antagonistic Activity (AU/mL)		
	ELY	NG1	NG2
MRSA	111.11±106.94	142.22±53.89	102.22±94.59
A. hydrophilia	1.78±3.079	26.67±26.67	26.67±26.67
A. baumanii	0.44±0.77	17.78±20.37	26.67±26.67
Candida	102.22±40.73	97.78±33.55	111.11±33.55
Salmonella	97.78±46.83	120.00±26.67	128.89±33.55
E. coli	142.22±60.12	93.33±26.67	266.67±120.00
E. cloacae	0.4±0.77	31.11±27.76	160.00±183.30
E. aerogenes	1.33±1.33	44.44±40.73	48.89±46.83
Pseudomonas	35.56±33.55	31.11±33.55	40.00±48.07
K. pneumoniae	151.11±68.42	113.33±43.72	146.67±35.28
MDR K. pneumoniae	71.11±73.43	100.00±50.33	120.00±53.33
R. ornithinolytica	84.44±94.59	35.56±33.55	120.00±61.10
S. odorifera	26.67±26.67	9.33±15.03	13.33±23.09
P. mirabilis	40±35.28	13.33±23.09	8.89±15.40
Average	**61.87±53.77**	**41.61±45.91**	**94.29±72.13**

AU/mL = Arbitrary unit per mL.

Table 4.8: Antibacterial Activity of LAB Isolates and Penicillin against the Indicator Bacterial Pathogens following Agar-Well Diffusion Method

Pathogenic Bacteria	ZDI (mm) ± SD for Indicator Bacteria		
	ELY	NG1	NG2
MRSA	10.00±5.00[a]	8.33±7.64 [a]	10.33±1.53 [a]
A.hydrophilia	5.67±5.13 [a]	11.00±4.58[b]	0.33±0.58 [a]
A.baumanii	15.00±5.00[b]	1.43±2.23 [a]	6.67±7.02[ab]
Candida	-	15.00±2.00[b]	3.67±3.51 [a]
Salmonella	4.67±5.03 [a]	2.33±2.08 [a]	7.33±7.02 [a]
E. coli	19.00±2.65 [a]	24.67±4.51 [a]	24.33±14.01 [a]
E. cloacae	1.00±1.73 [a]	3.33±3.51 [a]	2.33±2.52 [a]
E. aerogenes	6.33±6.03 [a]	3.00±2.65 [a]	4.00±1.00 [a]
Pseudomonas	4.33±3.79 [a]	4.00±3.61 [a]	4.33±4.04 [a]
K. pneumoniae	6.67±7.02 [a]	10.33±6.43 [a]	10.33±4.16 [a]
MDR K.pneumoniae	1.67±2.89 [a]	21.00±7.55[b]	10.33±2.52 [a]
R. ornithinolytica	4.00±3.61 [a]	7.33±2.52 [a]	8.67±3.06 [a]
S. odorifera	8.33±8.02 [a]	2.67±2.52 [a]	6.00±2.65 [a]
P. mirabilis	1.67±2.89 [a]	1.00±1.73 [a]	13.33±6.11[b]

ZDI: Zone Diameter of Inhibition; SD: Standard Deviation; numbers followed by the same letter superscripts in the same row do not vary significantly by Duncan's multiple range test at $p < 0.05$

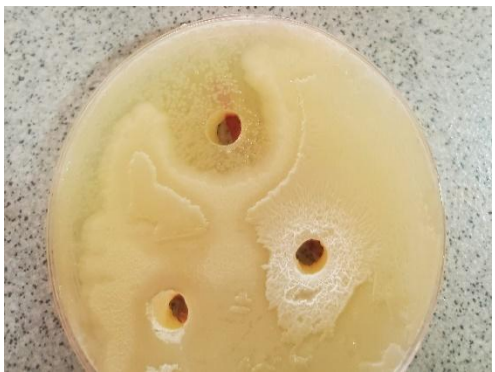
Table 4.9: Growth Inhibitory Activity of LAB and Penicillin expressed in "AU/mL" for Bacterial Pathogens.

Pathogenic Bacteria	Antagonistic Activity (AU/mL)		
	ELY	NG1	NG2
MRSA	133.33±66.67[a]	111.11±101.84[a]	137.78±20.37[a]
A.hydrophilia	75.56±68.42[ab]	146.67±61.10[b]	6.67±6.67[a]
A.baumanii	200.00±66.67[a]	19.11±29.70[b]	88.89±93.65[ab]
Candida	-	200.00±26.67 [b]	48.89±46.83 [a]
Salmonella	62.22±67.12 [a]	31.11±27.76 [a]	97.78±93.65 [a]
E. coli	253.33±35.28 [a]	328.89±60.12 [a]	324.44±186.82 [a]
E.cloacae	13.33±23.09 [a]	44.44±46.83 [a]	31.11±33.55 [a]
E. aerogenes	84.44±80.37 [a]	40.00±35.28 [a]	53.33±13.33 [a]
Pseudomonas	57.78±50.48 [a]	53.33±48.07 [a]	57.78±53.89 [a]
K.pneumoniae	88.89±93.65 [a]	137.78±85.72 [a]	137.78±55.51 [a]
MDR K.pneumoniae	22.22±38.49 [a]	280.00±100.66 [b]	137.78±33.55 [a]
R.ornithinolytica	53.33±48.07 [a]	97.78±33.55 [a]	115.56±40.73 [a]
S.odorifera	111.11±106.94 [a]	35.56±33.55 [a]	80.00±35.288 [a]
P.mirabilis	22.22±38.49 [a]	13.33±23.09 [a]	177.78±81.47 [b]
Average	**84.13±71.89**	**109.94±99.60**	**106.83±78.86**

AU/mL = Arbitrary Unit per mL. Numbers followed by the same letter superscripts in the same row do not vary significantly by Duncan's multiple range test at $p < 0.05$

Table 4.10: Antibiotics Susceptibility Test of Bacterial Pathogens

	Antibiotics								
Indicator Bacteria	**Gentamicin**	**Oflaxacin**	**Levofloxacin**	**Imipenem**	**Nitrofurantoin**	**Ceftriaxone**	**Augmentin**	**Cefexime**	**Penicillin**
MRSA	S	S	S	S	R	R	R	R	R
A.hydrophilia	S	S	S	S	S	R	R	R	R
A.baumanii	S	S	S	S	S	S	R	R	R
Salmonella	S	S	S	S	R	R	R	R	R
E. coli	S	S	S	S	R	S	R	R	R
E.cloacae	R	S	S	R	R	R	R	R	R
E. aerogenes	S	S	S	R	R	R	R	R	R
Pseudomonas	R	R	S	S	S	R	R	R	R
K.pneumoniae	S	S	S	S	R	R	R	R	R
MDR K.pneumoniae	R	R	R	R	R	R	R	R	R
R.ornithinolytica	R	R	S	R	R	R	R	R	R
S.odorifera	R	R	R	R	R	R	R	R	R
P.mirabilis	S	S	S	S	R	R	R	R	R



MRSA (NG2 + Penicillin) MDR *K. pneumoniae* (NG2 + Penicillin)

Plate 4.2: Agar-well diffusion technique demonstrating the synergestic combination of LAB isolates and Penicillin against 2 bacterial pathogens. The Zones of Inhibition is seen around the LAB and Penicillin mixtutre (injected into each hole) against 2 of the bacterial pathogens shown above (MRSA and *MDR K. pneumoniae*).

4.1.3 Results of Antibiotic Susceptibility Test of Bacterial Pathogens

Antibiotic susceptibility pattern of all 14 indicator strains against different antibiotics were studied. Results showed that MRSA was resistant to all tested Gram-positive antibiotics except four antibiotics (Levofloxacin, Gentamicin, Imipenem and Oflxacin)). MDR *K. pneumoniae*, *S. odorifera* and *R. ornithinolytica* were also resistant to all tested antibiotics, with only *R. ornithinolytica* being sensitive to Levofloxacin. *A. hydrophilia*, *Salmonella, E. coli*, *P. mirabilis* and *K. pneumonia* were all sensitive to Levofloxacin, Gentamicin, Imipenem, Oflaxacin; with *A. hydrophilia* and *E. coli* being additionally sensitive to Nitrofurantoin, and *E. coli* to Ceftriaxone. *E. cloacae* and *E. aerogenes* were both sensitive to Levofloxacin and Oflaxacin with *E. aerogenes* being additionally sensitive to Gentamicin. *Pseudomonas* was sensitive to Levofloxacin, Imipenem and Nitrofurantoin while *A. baumanii* was sensitive to all tested antibiotics except Augmentin and Cefexime.

All 14 bacterial pathogens were resistant to Penicillin which is as a result of the fact that most Gram-negative bacteria are β-lactamases which inactivates β-lactam drugs (Penicillin) by hydrolyzing a specific site in the β-lactam ring structure, causing the ring to open. The open-ring drugs are not then able to bind to their target PBP proteins[1].

4.1.4 Multiple Antibiotics Resistance (MAR) Index Calculation

Using the formula a/b, where 'a' denotes the number of antibiotics to which an isolate was resistant and 'b' denotes the total number of antibiotics tested, the MAR index was determined for each isolate, computed and interpreted in accordance with Krumperman[2].

4.11 MAR Index of Indicator Bacteria Isolates

Indicator bacteria	No of antibiotics to which isolate was resistant	MAR index = a/b
MRSA	5	0.56
A.hydrophilia	4	0.44
A.baumanii	3	0.33
Salmonella	5	0.56
E. coli	4	0.44
E.cloacae	7	0.78
E. aerogenes	6	0.67
Pseudomonas	6	0.67
K.pneumoniae	5	0.56
MDR K.pneumoniae	9	1.0
R.ornithinolytica	8	0.89
S.odorifera	9	1.0
P.mirabilis	5	0.56

The MAR index ranged from 0.33 to 1.0 with the average MAR index being 0.67 in two isolates.

The MAR index of all isolates examined in this investigation was greater than 0.2, suggesting a high-risk source of contamination when antibiotics are often used.

The most prevalent cause of multiple antibiotic resistance (MAR) in bacteria is the existence of plasmids that carry one or more resistance genes, each of which codes for a distinct antibiotic

resistance phenotype. The significant need for widespread, local antimicrobial resistance surveillance and the development of efficient therapies to lessen multidrug resistance in such organisms is shown by the high prevalence of multidrug resistance[3].

4.2 Discussion of Findings

4.2.1 Discussion of Antibacterial Activity of LAB Strains

By using a modified agar overlay and agar-well diffusion technique, *Leuconostoc mesenteroides*, *Weissella paramesenteroides*, *Limosilactobacillus fermentum* were observed to evaluate their antibacterial activity. To illustrate this point, the antagonistic impacts of the various LAB strains were tested against a variety of indicator microorganisms, including MRSA, *Aeromonas hydrophilia, Acinetobacter baumanii, Candida sp, Salmonella sp, Escherichia coli, Enterobacter cloacae, Enterobacter aerogenes, Pseudomonas aeruginosa, Klebsiella pneumoniae, MDR Klebsiella pneumoniae, Rauutella ornithinolytica, Serratia odorifera,* and *Proteus mirabilis*.

The "R" values of the isolated LAB isolates against the Gram-negative pathogenic bacteria have been represented in Table 4.4. The lowest "R" value (-2.17±0.58mm) was recorded due to the action of *Leuconostoc mesenteroides* against *P. mirabilis*, while the highest "R" value (7.33±0.76) was recorded due to the action of *Weissella paramesenteroides* against *R. ornithinolytica.* Ranging from 0.00±1.00 to 7.33±0.76 mm, were from the action of *W. paramesenteroides* against the pathogenic bacteria tested; *Limosilactobacillus fermentum* had "R" values -1.83±1.15 – 6.17±1.26 mm, and *L. mesenteroides* had -2.17±0.58 to 7.00±1.73 mm.

Following the agar overlay procedure, the LAB isolates showed high antibacterial activity against all indicator bacteria tested. *Weissella paramesenteroides* isolate had intermediate growth inhibitory activity against *MRSA*, *Candida, K. pneumoniae and R. ornithinolytica* having ZDIs

18.67±3.21, 19.00±2.00, 19.00±2.00 and 19.67±1.53 mm, respectively. *MRSA* showed the highest sensitivity to *Limosilactobacillus fermentum* (ZDI: 17.33±2.52 mm) while *P. mirabilis* showed lowest sensitivity to *Leuconostoc mesenteroides* (ZDI: 0.67±1.15mm). In comparison to other isolates, *Weissella paramesenteroides* demonstrated moderate activity against all indicator bacterial strains, with ZDIs ranging from 2.67±2.52 to 19.67±1.53 mm compared to other isolates as illustrated in Table 4.5.

Although the levels of antagonistic effects varied across the LAB genus, all LAB strains examined had positive effects on all of the tested indicator microorganisms. The antagonistic activity, following agar-well diffusion (based on the ZDIs values obtained around the culture filtrate-loaded wells on the agar plates), of the LAB isolates against the indicator bacteria is represented in Table 6. The results revealed that the *L. mesenteroides* had highest activity, with ZDI of 23.67 ± 1.53 mm, against *K. pneumoniae*, while *E. cloacae, E. aerogenes, Pseudomonas* and *P. mirabilis* had no recorded sensitivity to *L. fermentum*. *E. coli* was highly sensitive to *L. mesenteroides*, having ZDIs 20.00±9.00 mm.

Among the LAB isolates, *L. mesenteroides* had lowest activity (0.44±0.77AU/mL), among the pathogenic indicator bacterial isolates, while the top level of growth inhibitory components produced was 266.67±120.00AU/mL, by *L. fermentum*, and the values ranged from 9.33±15.03 to 142.22±53.89 AU/mL, for *W. paramesenteroides*, and from 0.44±0.77 to 151.11±68.42 AU/mL, for ELY (Table 4.7). For *L. mesenteroides, E.coli* demonstrated the highest inhibitory activity at 142.22±60.12, while *A. baumanii* and *E. cloacae* exhibited the lowest inhibitory activity at 0.44±0.77. MRSA displayed the highest inhibitory activity among *W. paramesenteroides* at 142.22±53.89, whereas *S. odorifera* had the lowest inhibitory activity at 9.33±15.03. Among the pathogenic bacteria that *L. fermentum* inhibited, *E. coli* demonstrated the

highest inhibitory activity at 266.67±120.00, while *P. mirabilis* exhibited the lowest inhibitory activity at 8.89±15.40. *L. fermentum* (94.29±72.13) has the highest mean growth inhibitory activity.

The antagonistic activity, following agar-well diffusion of the LAB isolates combined with Penicillin against the indicator bacteria is represented in Table 4.8. The results revealed that the *E. coli* was highly sensitive to all the LAB with *L. mesenteroides* + Penicillin, having ZDIs 19.00±2.65mm, *W. paramesenteroides* + Penicillin having ZDIs 24.67±4.51mm and *L. fermentum* + Penicillin having ZDI 24.33±14.01mm (Table 4.8). *Candida* had no recorded sensitivity to *L. fermentum* +Penicillin.

Among the LAB combined with Penicillin, *L. fermentum* + Penicillin had the lowest (6.67±6.67AU/mL), and highest (324.44±186.82AU/mL) activity of growth inhibitory components produced among the pathogenic indicator bacterial isolates. The values ranged from 13.33±23.09 to 328.89±60.12 AU/mL, for *W. paramesenteroides* + Penicillin, and from 13.33±23.09 to 253.33±35.28 AU/mL, for *L. mesenteroides* + Penicillin (Table 4.9). For all the LAB isolates + Penicillin, *E.coli* demonstrated the highest inhibitory activity at 253.33±35.28 (*L. mesenteroides* + Penicillin), 328.89±60.12 (*W. paramesenteroides* + Penicillin), and 324.44±186.82 (*L. fermentum* + Penicillin). *W. paramesenteroides* + Penicillin (109.94±99.60) has the highest mean growth inhibitory activity.

4.2.2 Discussion

An exceptional barrier against bacterial infections is provided by the skin. Despite the fact that many bacteria come into contact with or live on the skin, they typically cannot cause an infection. When bacterial skin infections do arise, any part of the skin can be affected, whether a small

patch or the full surface. They may be innocuous or potentially fatal, depending on their severity. Relatively minor infections can include Folliculitis, Impetigo and small skin abscesses, and more serious bacterial skin infections could include Cellulitis, Large skin abscesses and wound infections all these disruptions can lead to pain, swelling, systemic symptoms or most importantly, Skin and soft tissue infections[3].

Most Skin infections are caused by bacteria, specifically *Staphylococcus, Streptococcus* and other opportunistic pathogenic Gram-negative bacteria. This study aims to evaluate the potential of combining Lactic acid bacteria cell extract and antibiotics as a treatment strategy for skin infections and assess the effectiveness of this combination together on infections caused by Methicillin-resistant *Staphylococcus aureus* (MRSA) and other opportunistic bacteria including A. *hydrophilia, A. baumanii, Candida, Salmonella, E. coli, E. cloacae, E. aerogenes, Pseudomonas, K. pneumoniae, MDR K. pneumoniae, R. ornithinolytica, S.odorifera,* and *P.mirabilis* and evaluation of resistance of these bacteria to antibiotics.

Weissella is a genus earlier considered a member of the family *Leuconostocaceae,* which was reclassified into the family *Lactobacillaceae* in 1993. The *Weissella* genus has begun to take center stage in the past few years owing to its probiotic potential and its many prospective applications, ranging from the healthcare industry to the skin care and food industries. Due to its antimicrobial potential against other pathogenic microorganisms including but not limited to *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhi,* and *Salmonella enterica*. The only limitation to its widespread use is the lack of a significant volume of research at the moment and a handful of reported cases of pathogenicity. However, a bulk of these cases are a result of some preexisting disposition or comorbidity associated with the host[4].

W. paramesenteroides is reported to be heat-resistant and show activity against a range of Gram-positive bacteria. The cell extract from *W. paramesenteroides* was classified into class II and predicted to affect the integrity of plasma membranes of pathogens, causing an efflux of required nutritious cellular metabolites, thereby resulting in cell death[5].

The genus of *Lactobacillus* has grown very large and diverse. Therefore, the taxonomy has recently been updated, thus introducing new genus names. Based on physiology and genomics, *Lactobacillus fermentum* was therefore re-categorised as *Limosilactobacillus fermentum*. It still remains within the family of *Lactobacillaceae*[6].

A study showed that, out of the 2 different strains of *Lactobacillus* tested for antibacterial activity, *L. fermentum* appeared to be more efficient than the *L. brevis* strain tested against bacterial pathogens[8]. Two *L. fermentum* strains isolated from the oral mucosa of chickens inhibited the growth of *S. aureus* strains including three methicillin resistant strains. The strain of *L. fermentum* isolated from dairy milk inhibited *Listeria monocytogenes*, *E.coli* and *S. aureus*, indicating the presence of strong antimicrobial activity in different strains of *L. fermentum*[7].

The *Leuconostoc* genus belongs to Firmicutes phylum, which includes Gram-positive, heterofermentative (fermenting glucose to D-lactic acid, ethanol/acetic acid, and CO2 from phosphoketolase pathway) microorganisms, and presents coccoid or rod-like shape[8]. In a study, results showed the inhibitory activity of *Leuconostoc mesenteroides* biofilm against *Staphylococcus aureus* by using different concentrations of the biofilm thereby increasing the inhibition zones again *S. aureus*[9] .Consequently, the study also showed antibacterial activity of *L. mesenteroides* against *E.coli* and *Pseudomonas aeruginosa*, and the effectiveness of the biofilm against these organisms was reported to may have been due to metabolic products of *Leuconostoc mesenteroides* such as organic acids , diacetyl, CO2,hydrogen peroxide , and

secondary metabolites (such as bacteriocin). Many *Leuconostoc mesenteroides* species produce many organic acids in addition to a group of antimicrobial compounds, especially protein products called bacteriocines (such as Carnosin and Leuconocin)[9].

Following the agar overlay procedure, the *Lactobacilli* isolates displayed significant antibacterial activity against all indicator bacteria tested. Notably, *W. paramesenteroides* exhibited intermediate growth inhibitory activity against MRSA, *Candida, K. pneumoniae*, and *R. ornithinolytica,* as evidenced by ZDIs (Zone of Inhibition). Of particular interest is the heightened sensitivity of MRSA to *Limosilactobacillus fermentum*, indicating its potent inhibitory effect. Conversely, *L. mesenteroides* displayed the lowest sensitivity against *P. mirabilis* (Table 4.5). This reveals significant variations in growth inhibitory activities among the different LAB isolates, highlighting the diverse responses of these microorganisms to inhibitory components and these emphasizes the diverse capabilities of these *Lactobacilli* to produce inhibitory components, *Limosilactobacillus fermentum* standing out as a particularly potent inhibitor. Such variations in growth inhibitory activities could have significant implications for understanding bacterial interactions, antimicrobial potential, and their possible applications in various fields, including medicine and biotechnology.

In contrast to the findings from LAB isolates used alone, this study observed significantly enhanced antibacterial activity when LAB isolates were combined with penicillin against all tested pathogenic bacteria. Remarkably, all LAB isolates and penicillin combinations demonstrated robust growth inhibitory effects, particularly against *MRSA, K. pneumoniae, and E. coli*, as indicated by substantial ZDI results (Table 4.8 & 4.9). Given the common issue of antimicrobial resistance rendering single-antimicrobial treatments less effective, this study parallels the work of Kang, who investigated a novel approach[10]. They examined a combination

formulation comprising two bacteriocins, garvicin KS and micrococcin P1, along with penicillin G for the treatment of MRSA skin infections in a mouse model. Their findings, compared with the conventional use of fucidin cream for skin infections, were noteworthy. The three-component formulation successfully eliminated *S. aureus* Xen31, a multidrug-resistant MRSA strain, from skin puncture wounds over four consecutive days of treatment[11]. These results suggest a promising avenue in the battle against antibiotic resistance. The inclusion of bacteriocins in combination with antibiotics like penicillin G may potentially rejuvenate the effectiveness of these antibiotics, providing a novel strategy to combat challenging infections.

The data presented in this research implies that cell free supernatant from LAB may be useful for treating Gram negative and multi-drug resistant infections.

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Chapter Five

Conclusion

5.1 Summary of Findings

With regards to treating skin infections, we discovered that a combination of the two antimicrobials (different strains of LAB and Penicillin) were more effective not only against MRSA and MDR *K. pneumoniae* but also against a variety of other Gram-negative opportunistic pathogens and Candida reportedly found in skin and soft tissue infections. This finding illustrates the combination's broad therapeutic value. For MRSA and MDR *K. pneumoniae*, none of the two antimicrobials mentioned above had very potent effect when used alone, as compared to when used in combination with Penicillin, an antibiotics that has been known to be resistant to a lot of bacteria. Therefore, it is a healthy development to learn that the combination made MRSA, MDR *Klebsiella pneumoniae*, *Candida* and other bacteria strains such as *Raoultella ornithinolytica*, *Serratia odorifera* sensitive to PenG, which were otherwise useless against these organisms when administered alone. We could hypothesize that the formulation causes PBP-2a to become inactive through an unidentified protein-level or gene regulation mechanism, and that this inactivation makes MRSA susceptible to PenG once more.

This study aims to demonstrate that the correct bacteriocins may be used in the right combinations with other antimicrobials to create effective medication formulations that are not only very active against pathogens but also stop the emergence of resistance. Most bacteriocins differ from conventional antibiotics in their modes of action, making them excellent candidates for next-generation antimicrobials or at the very least crucial additions to the antimicrobial armory to combat bacteria that are resistant to conventional antibiotics.

5.2 Conclusion

This study showed that these three (*Weissella paramesenteroides, Limosilactobacillus fermentum, Leuconostoc mesenteroides*) LAB strains contained a broad-spectrum antibacterial activity against MRSA, Candida and Gram negative pathogenic bacterial strains with an inhibition zone ranging from 5 to 20 mm following both Agar-well diffusion method and Agar overlay method. These LAB strains had better results when used in combination with an antibiotic (Penicillin) with inhibition zone ranging from 5mm to 40mm following agar- well diffusion method. The research findings therefore demonstrated that the LAB isolated from freshly fermented milk products from Ibadan, Nigeria were a rich source of metabolites with antibacterial properties and when used in combination with powerful antibiotics, the effect becomes stronger and much more effective. As a result, the identification and characterization of LAB from fermented milk products from different geographical regions in Oyo state may serve as a useful source for the discovery of new and chemically diverse compounds with a variety of antibacterial, antifungal and probiotic functions used alone or in combination.

However, more research is needed to determine whether the antibacterial essence of these native Lactobacilli might be exploited as an alternative treatment to cure infections and battle bacterial antibiotic resistance.

5.3 Recommendations

Due to the characteristics of bacteriocins, a wide range of medical applications are possible. As a result, bacteriocin safety demands a lot of focus and attention and although bacteriocins' antimicrobial action has been extensively explored, the following recommendations should be considered:

1. It is vital to look at their immunogenicity and toxicity both in vitro and in vivo as there is a lack of available data regarding the in vitro and in vivo safety of bacteriocins.
2. Regulations put out by the federal agencies of the department of health for all countries must be properly adhered to, to obtain legal approval of bacteriocins as trustworthy and safe medicinal agents,
3. Many experiments are still to be carried out to evaluate their cytotoxicity in eukaryotic cells, ability to cause cell death, growth inhibitory effect, hemolytic activity, acute and subchronic toxicity.
4. Lastly, the primary goal should not be to increase the treatment toolbox but rather to provide alternative methods that do not directly involve the use of antibiotics, hence minimizing the impacts and consequences of multi-drug resistance organisms on the general populace.

5.4 Contributions to Knowledge

In pursuit of innovative solutions to the pressing challenge of drug-resistant skin pathogens, this study uncovers several significant contributions to our understanding and treatment of these infections.

Our contributions to knowledge encompass the following key aspects:

i. **Novel Therapeutic Approach**: This research contributes to knowledge by introducing an approach in the field of dermatology and infectious diseases. The exploration of bacteriocin-antibiotic synergy represents a strategy for combating drug-resistant bacterial strains commonly associated with skin infections.

ii. **Synergy Understanding**: This study deepens our understanding of how bacteriocins and antibiotics can work synergistically to enhance their antibacterial effects. By elucidating the mechanisms underlying this synergy, we provide valuable insights into the potential for combination therapy against drug-resistant skin pathogens.

iii. **Optimal Combinations**: Our research investigates and identifies the optimal combinations of specific bacteriocins and antibiotics that exhibit synergistic action against resistant bacterial strains. This knowledge is essential for developing precise and targeted treatment regimens, optimizing therapeutic outcomes, and minimizing adverse effects.

iv. **In Vivo Efficacy and Safety**: By assessing the in vivo efficacy and safety of combining bacteriocins and antibiotics, this research bridges the gap between in vitro studies and clinical applications. This contribution informs healthcare practitioners about the practicality and potential benefits of this treatment approach in living organisms.

v. **Microbiota Impact**: We shed light on the impact of bacteriocin-antibiotic combination therapy on the normal skin microbiota. Understanding how this treatment affects beneficial skin microbes contributes to a holistic approach to skin infection management, aiming to minimize disruptions to the skin's natural defenses.

vi. **Resistance Development Awareness**: This research raises awareness of the potential for resistance development to bacteriocins and antibiotics in the presence of each other. By studying the evolution of resistance mechanisms, we provide essential insights for mitigating the risks associated with long-term therapeutic use.

vii. **Future Therapeutic Potential**: The findings of this research open avenues for future investigations and therapeutic development. They encourage further research into the

development of enhanced bacteriocins, antibiotics, and combination therapies with even greater synergistic action against drug-resistant skin infections.

5.5 Suggested Areas for Further Research

1. Additional research is needed to investigate and identify additional bacteriocin-antibiotic combinations that exhibit high levels of synergy against drug-resistant skin pathogens. This can expand the range of treatment options and potentially overcome resistance issues.
2. Rigorous clinical trials and studies involving human subjects should also be conducted to validate the in vivo efficacy and safety of bacteriocin-antibiotic combination therapy for various skin infections, evaluating the treatment's real-world effectiveness and potential side effects.
3. Researching the development of novel bacteriocins with enhanced properties, such as increased specificity, broader antimicrobial activity, and improved stability, can be developed to further optimize combination therapies.
4. The investigation for the potential for personalized medicine in the context of bacteriocin-antibiotic combination therapy is an approach that should be given closer attention as personalized medicine is developing. Individual variations should be assessed in response to specific combinations and tailor treatments accordingly.
5. Long-term outcomes of patients treated with bacteriocin-antibiotic combinations, with a focus on relapse prevention should also be researched on, which is examining the durability of treatment effects and the potential for recurrent infections.
6. Lastly, cost-benefit analyses should be conducted in diverse healthcare settings to assess the economic impact of implementing bacteriocin-antibiotic combination

therapy, while also considering factors such as hospitalization costs, reduced recurrence rates, and healthcare resource allocation.

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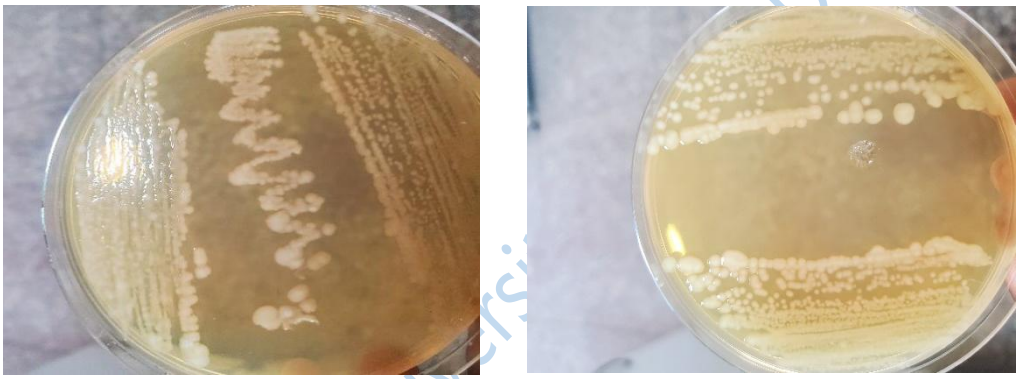
Appendices

Appendix I

Isolation and Growth of LAB Isolates

Serial dilutions of traditionally fermented milk were made up to 10^{-9} using normal (0.85% NaCl w/v) saline solution. 0.1 mL of dilutions 10^{-3}, 10^{-5}, 10^{-7} was taken and spread on already prepared and sterilized De Man Rogosa and Sharpe (MRS) agar medium (Sterilized at 121°c for 15 minutes) with the help of a bent glass spreader. All plates were incubated in triplicates at 30°c for 48–72 h in an anaerobic jar. Representative pure colonies were subcultured on MRS agar and kept at 4°C for further analysis and characterization tests.



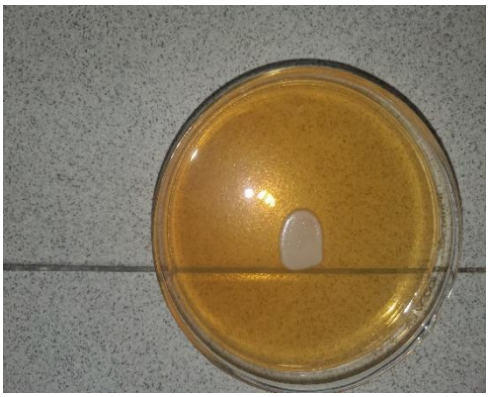
Pure cultures of LAB isolates, procured from traditionally fermented milk samples

Appendix II

Spot Inoculation of LAB

Spot inoculation of LAB isolates onto MRS agar plates were done by using a loop-full (≈10^5 CFU/spot) of fresh MRS broth culture of the LAB isolates which will have been previously at 35°C for 48 h, and the inoculated plates incubated at 37°C for 48 h. The MRS agar plates containing the growth of LAB in spot form (5 mm diameter) is thereafter overlaid with soft

Mueller Hinton agar (containing only 0.8% of bacteriological agar) pre-mixed with 10^8 CFU of the bacterial indicator strains (One on each agar overlay plate).



Appendix III

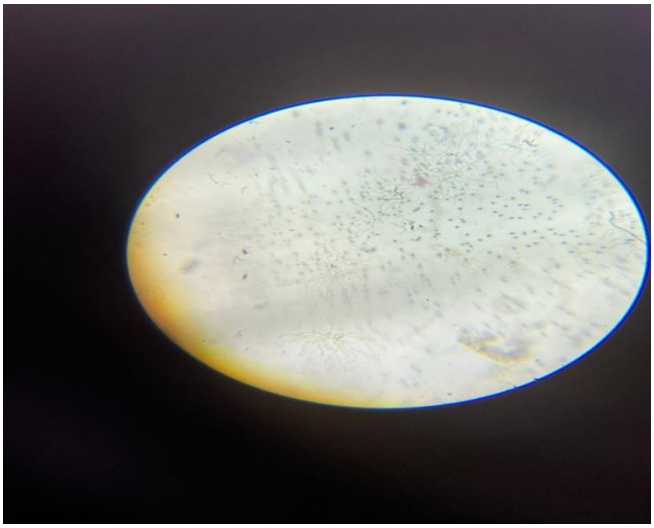
Gram Staining

A slide of the LAB sample to be stained is heat fixed by carefully passing the slide with a drop or small piece of the sample on it through a Bunsen burner three times. Cells of the LAB isolates are stained with crystal violet dye and left for about 1 minute before rinsing under gentle stream of water for about 5 seconds to remove crystal violet. Next, a Gram's iodine solution (iodine and potassium iodide) which is a mordant, is added for 1 minute to form a complex and fix the crystal violet to the bacterial cell wall.

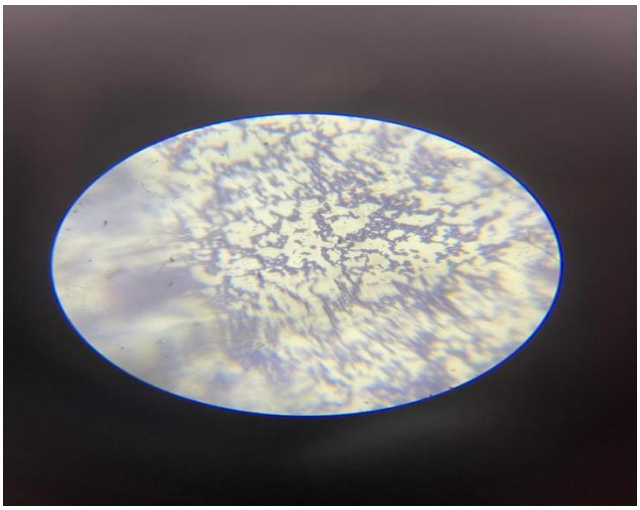
A decolorizer such as ethyl alcohol or acetone is added to rinse the glass slide for about 3 seconds and rinsed with a gentle stream of water. This dehydrates the peptidoglycan layer, shrinking and tightening it. The large crystal violet-iodine complex is not able to penetrate this tightened peptidoglycan layer, and is thus trapped in the cell in Gram positive bacteria. Conversely, the outer membrane of Gram-negative bacteria is degraded and the thinner

peptidoglycan layer of Gram-negative cells is unable to retain the crystal violet-iodine complex and the color is lost.

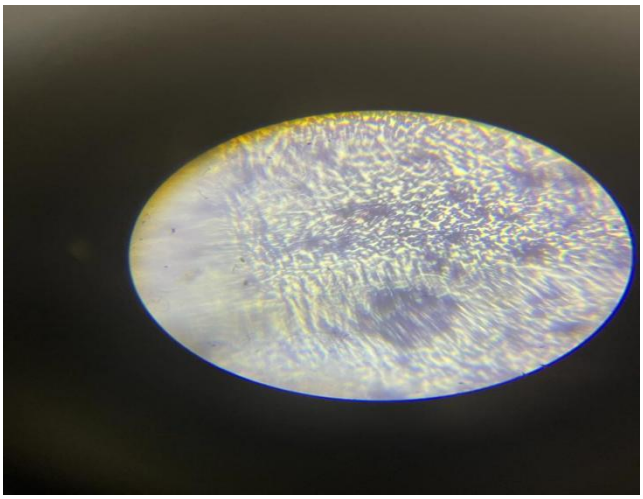
A counterstain, such as the weakly water-soluble safranin, is added to the glass slide for 1 minute and rinsed, staining it red. Since the safranin is lighter than crystal violet, it does not disrupt the purple coloration in Gram positive cells. However, the decolorized Gram-negative cells are stained red. The slide is then viewed under the microscope.



(NG2) *Lactobacillus fermentum* showing as Gram positive Cocci under the microscope



(NG1) *Weissella paramesenteroides* showing as Gram positive Rod under the microscope



(ELY) *Leuconostoc mesenteriodes* showing as Gram positive Rod under the microscope

Appendix IV

Measurement of Turbidity and Optical Density using Spectrophotometer

The spectrophotometer is turned on and wavelength is set at 600nm. The zero control is adjusted and % transmittance is set to 0%. The cuvette is then wiped with a sterile cloth to remove any liquid or finger prints, filled with about 4ml of sterile water serving as control blank and placed into the sample compartment and closed. The readings of the blank are read and noted. To standardize the instrument, the % transmission is set to 100. The cuvette is removed from the sample folder, emptied, filled with an inoculated LAB broth and thoroughly mixed, placed into the sample compartment and covered. The readings displayed on the scale is read and recorded and repeated for all other samples.

Results interpretations:

Calculate the absorbance (O.D.) for all the inoculated tubes by applying the formula:

Absorbance= -log(%T)/100

Plot the readings in terms of absorbance versus the time at which readings were taken.

It will be observed that the turbidity of some inoculated tubes increases with the incubation period, the O.D. would be found to increase while % T would be found to decrease specifying more growth of the cell population in the culture with increased incubation period.

Optical Density/Turbidity Measurement

Wavelength – 540nm

	Optical Density (OD)
Blank	0.486
10^{-1}	0.723
10^{-2}	0.561
10^{-3}	0.495
10^{-4}	0.485
10^{-5}	0.326
10^{-6}	0.193
10^{-7}	0.319

Appendix V

Plate Count

Serial dilutions (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-7}) of previously isolated LAB in MRS broth is made and plated. The population density of the LAB isolates is estimated by plating 2 drops of MRS broth containing LAB isolate growth into MRS agar plate and spreading the broth widely around the plate with the use of a sterile spreader and incubating at 37°c for 48. The number of bacteria colonies are counted.

Plate Count of LAB Isolates

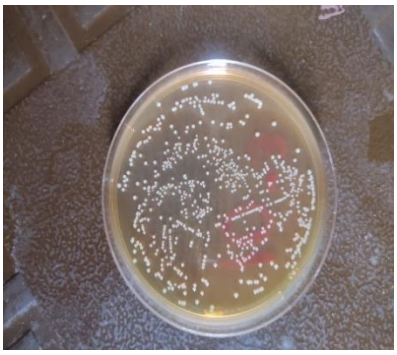
10^{-2} – 2,380 colonies

10^{-3} – 700 colonies

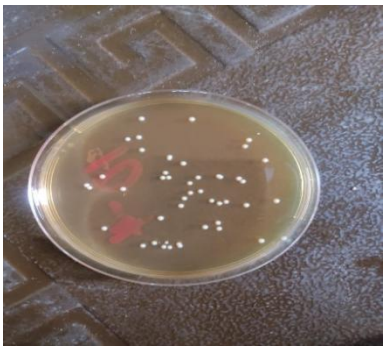
10^{-4} – 47 colonies

10^{-5} – 4 colonies

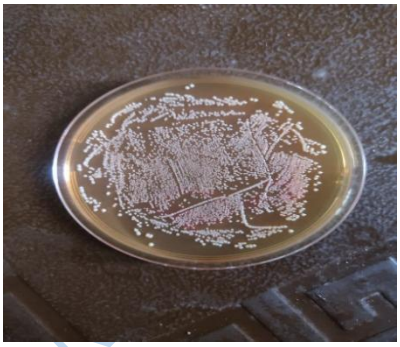
10^{-7} – 0 colonies



10^{-3} Plate Count



10^{-4} Plate Count



10^{-2} Plate Count

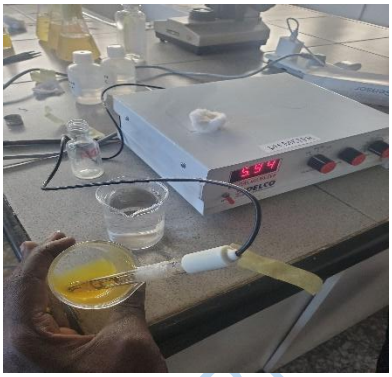
Appendix VI

Determination of pH of LAB Isolates as Quality Control Test using pH Meter

Small amount of fresh pH buffers was poured in beakers for calibration and the tip of the pH electrode is rinsed with distilled water and blotted dry with soft tissue. The pH electrode is then calibrated with pH buffers. After calibration, the pH of the LAB isolates is measured at room temperature (20°c – 25°c) by firstly dispensing LAB broth culture into a beaker and dipping the tip of the round pH electrode into the broth culture to measure the pH, making sure the tip touches the bottom of the broth culture medium with no gap between them. The pH displayed on the meter is recorded once reading is stable. The tip of the pH electrode is rinsed with distilled water and blot dry before measuring another sample and samples are discarded after testing. To obtain accurate results, pH buffers and LAB samples are at the same temperature. LAB samples were then adjusted with 1M NaOH or 1M HCl (if necessary) before dispensing for sterilization.

NG2 showing a pH of 5.70

ELY showing a pH of 5.78



NG1 showing a pH of 5.94

Appendix VII

Genetic Sequence of LAB Isolates after Partial Gene Amplification

>Genetic Sequence of (NG1) *Weissella paramesenteroides*

CTAATACATGCAAGAGAACGCTTTGTCTTTAANTGATNTGANGAGCTTGCTCTGATT
TGATTTTATCTGACAAAGAGTGGCGAACGGGTGAGTAACACGTGGGTAACCTACCC
CTTAGCAGGGGATAACATTTGGAAACAAGTGCTAATACCGTATAATACCAACAACC
GCATGGTTGTTGGTTGAAAGATGGTTCTGCTATCACTAAGAGATGGACCCGCGGTGC
ATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAATGATGCATAGCCGAGTTGA
GAGACTGATCGGCCACAATGGGACTGAGACACGGCCCATACTCCTACGGGAGGCAG
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AGCCGCGGTAATACGTATGTTCCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAG
CGCAGACGGTTATTTAAGTCTGAAGTGAAAGCCCTCAGCTCAACTGAGGAATGGCTT
TGGAAACTGGATGACTTGAGTGCAGTAGAGGAAAGTGGAACTCCATGTGTAGCGGT
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GCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAG
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GCCAACCCGCGAGGGTGCGCTAATCTCTTAAAGTATGTCTCAGTTCGGATTGTAGGC
TGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAATCGCGGATCAGCACGCCGCG

GTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAAC
ACCCAAAGCCGGTGGGGTAACCTTTT

>**Genetic Sequence of (NG2)** ***Lactobacillus fermentum***

CCTACATGCAAGTTGAACGCGTTGGCCCAATTGATTGATGGTGCTTGCACCTGATTG
ATTTTGGTCGCCAACGAGTGGCGGACGGGTGAGTAACACGTAGGTAACCTGCCCAG
AAGCGGGGGACAACATTTGGAAACAGATGCTAATACCGCATAACAACGTTGTTCGC
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ATTAGCTTGTTGGTGGGGTAACGGCCTACCAAGGCGATGATGCATAGCCGAGTTGA
GAGACTGATCGGCCACAATGGGACTGAGACACGGCCCATACTCCTACGGGAGGCAG
CAGTAGGGAATCTTCCACAATGGGCGCAAGCCTGATGGAGCAACACCGCGTGAGTG
AAGAAGGGTTTCGGCTCGTAAAGCTCTGTTGTTAAAGAAGAACACGTATGAGAGTA
ACTGTTCATACGTTGACGGTATTTAACCAGAAAGTCACGGCTAACTACGTGCCAGCA
GCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGAGAG
TGCAGGCGGTTTTCTAAGTCTGATGTGAAAGCCTTCGGCTTAACCGGAGAAGTGCAT
CGGAAACTGGATAACTTGAGTGCAGAAGAGGGTAGTGGAACTCCATGTGTAGCGGT
GGAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTACCTGGTCTGCA
ACTGACGCTGAGACTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGT
CCATGCCGTAAACGATGAGTGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGGA
GCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAG
GAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGC
GAAGAACCTTACCAGGTCTTGACATCTTGCGCCAACCCTAGAGATAGGGCGTTTCCT
TCGGGAACGCAATGACAGGTGGTGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTT
GGGTTAAGTCCCGCAACGAGCGCAACCCTTGTTACTAGTTGCCAGCATTAAGTTGGG
CACTCTAGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGACGACGTCAGATC
ATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAACGAGTC
GCGAACTCGCGAGGGCAAGCAAATCTCTTAAAACCGTTCTCAGTTCGGACTGCAGG
CTGCAACTCGCCTGCACGAAGTCGGAATCGCTAGTAATCGCGGATCAGCATGCCGC
GGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAA
CACCCAAAGTCGGTGGGGTAACCTTTT

>**Genetic sequence (ELY)** ***Leuconostoc mesenteriodes***

AACTAATACATGCAAGTCGAACGCACAGGGAAAGGTGCTTGCACCTTTCAAGTGAG
TGGCGAACGGGTGAGTAACACGTGGACAACCTGCCTCAAGGCTGGGGATAACATTT
GGAAACAGATGCTAATACCGAATAAAACTTAGTGTCGCATGACACAAAGTTAAAAG
GCGCTTCGGCGTCACCTAGAGATGGATCCGCGGTGCATTAGTTAGTTGGTGGGGTAA
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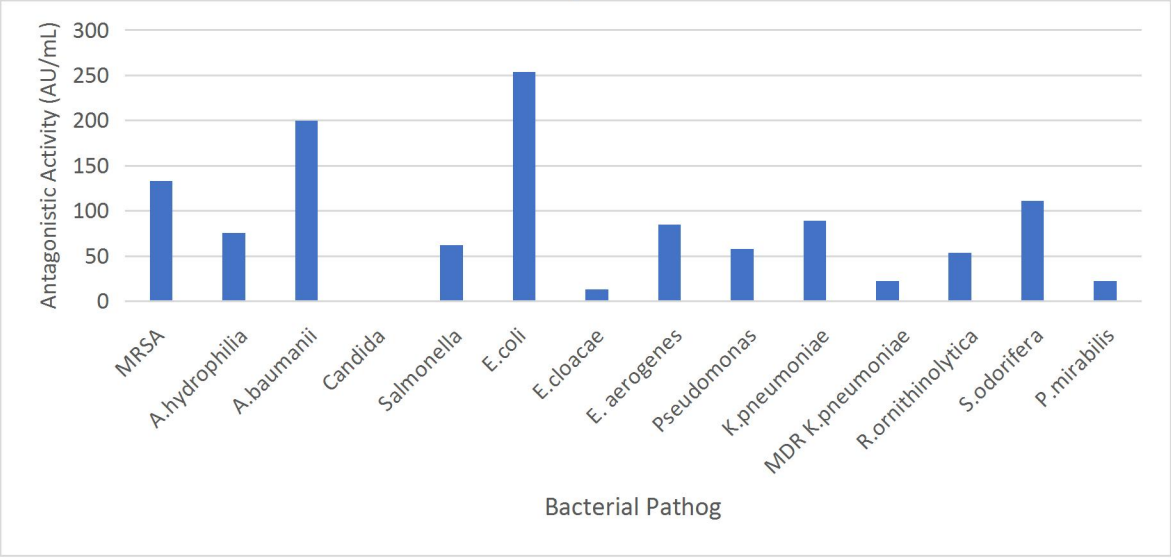
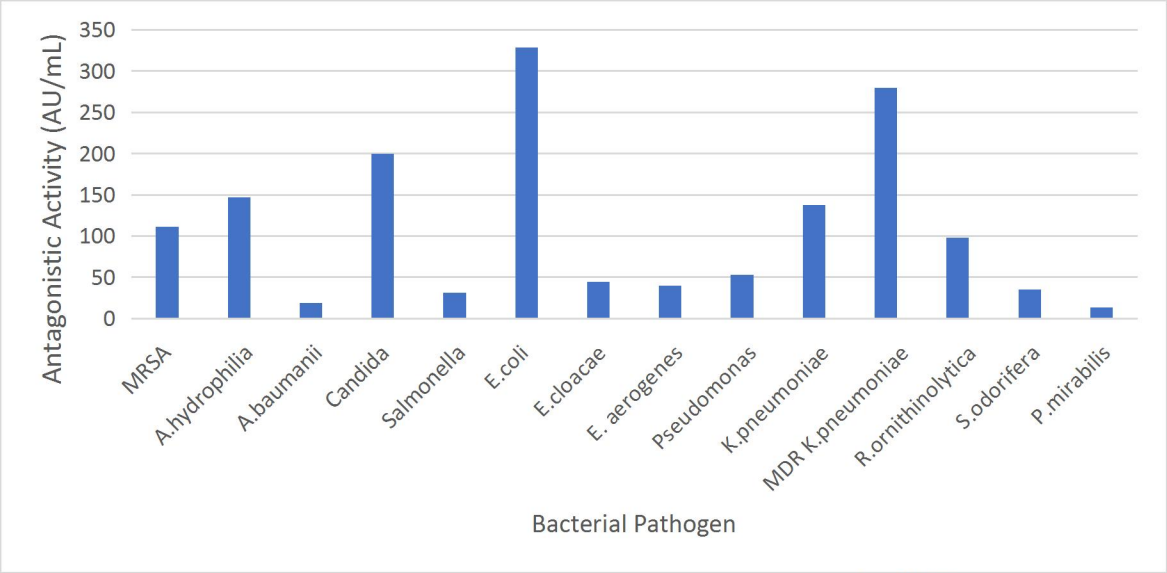
Appendix VIII

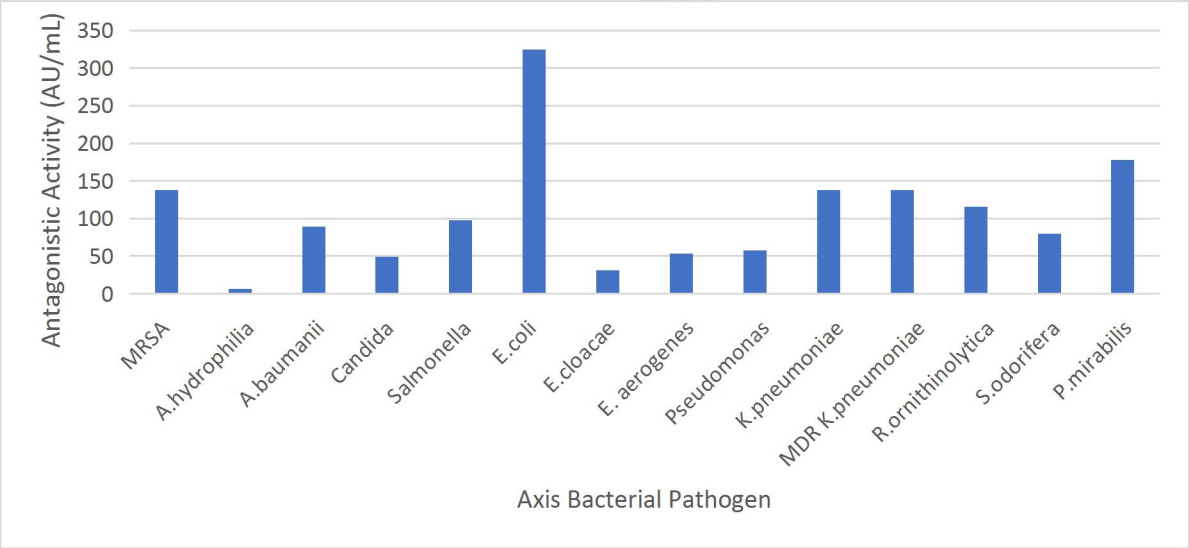
Graphical Representation of the Antagonistic Activity Results



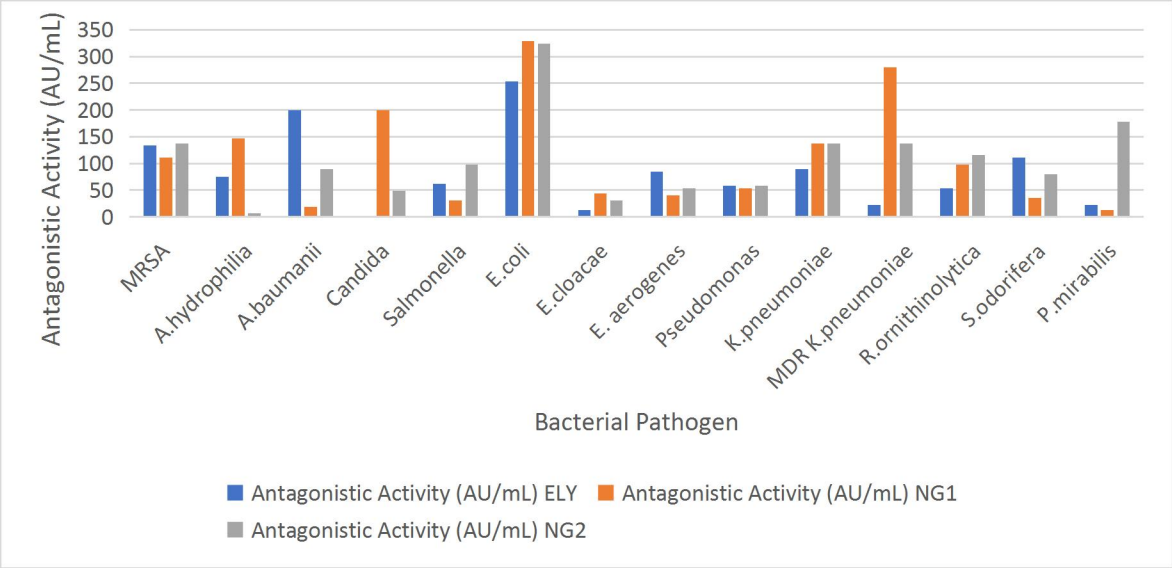
Growth Inhibitory Activity of LAB Isolate (ELY) Expressed in "AU/mL" for Bacterial Pathogens.



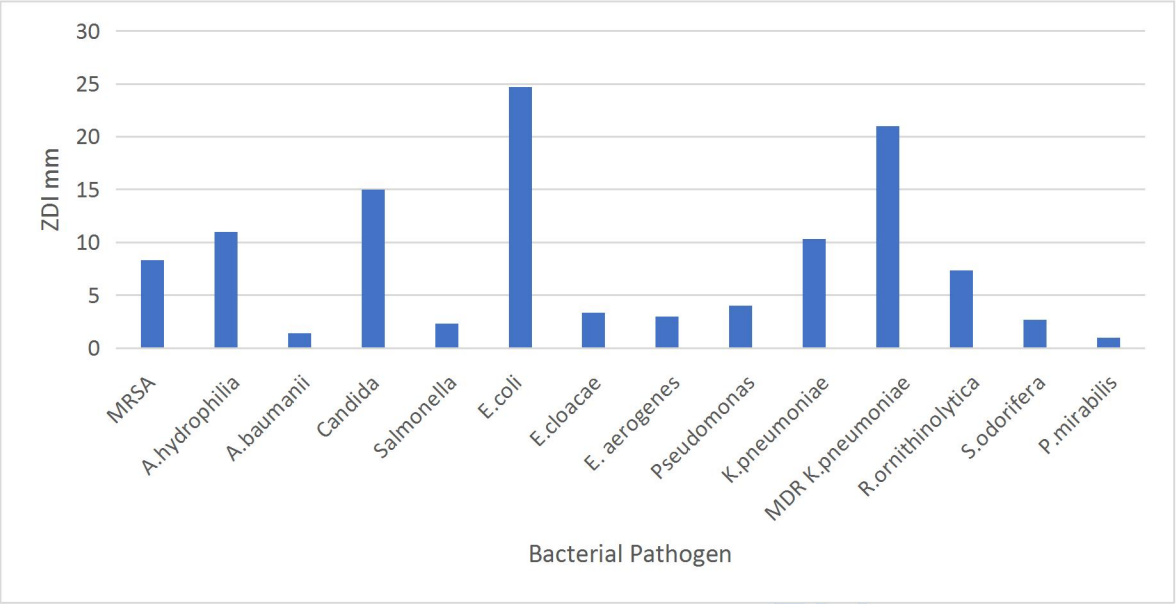
Growth Inhibitory Activity of LAB Isolate (NG1) Expressed in "AU/mL" for Bacterial Pathogens.



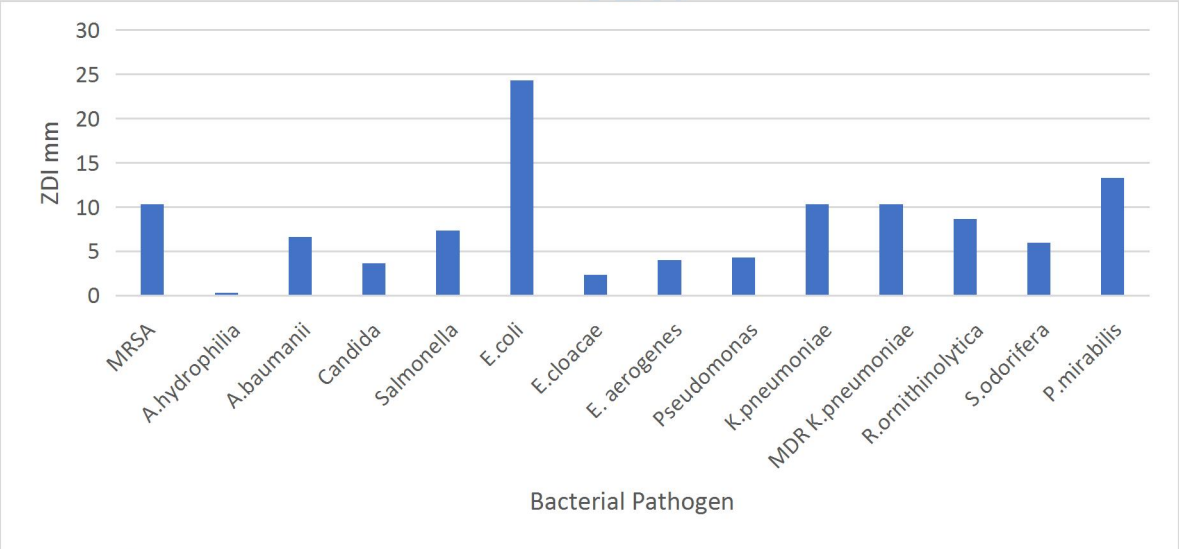
Growth Inhibitory Activity of LAB Isolate (NG2) Expressed in "AU/mL" for Bacterial Pathogens.



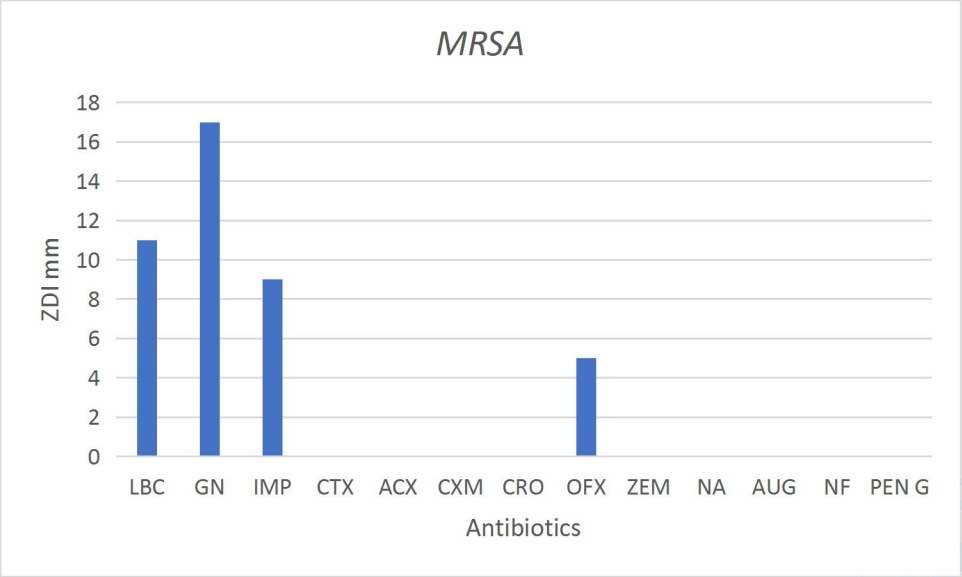
Growth Inhibitory Activity of LAB Isolates and Penicillin expressed in "AU/mL" for Bacterial Pathogens.



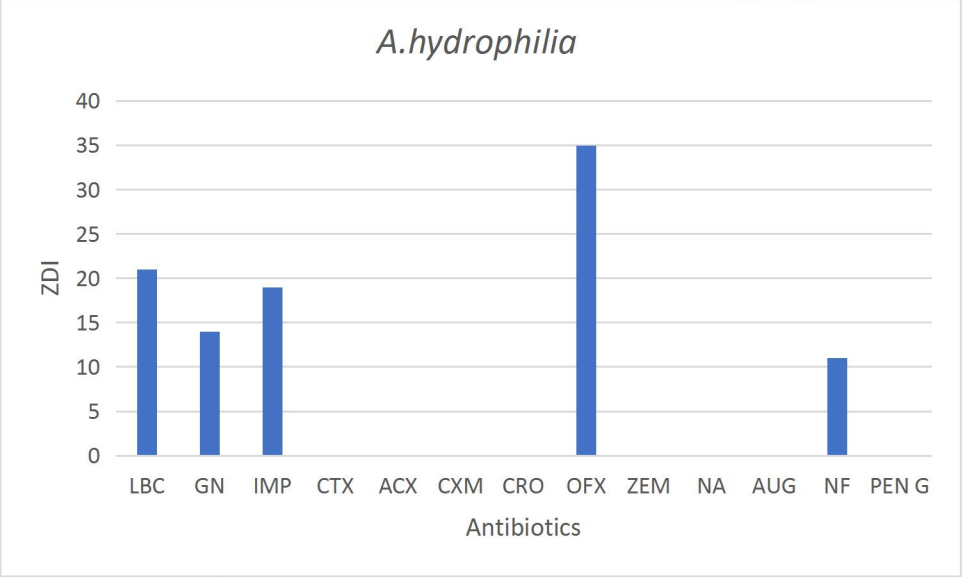
Antibacterial Activity of LAB isolates (NG1) and Penicillin against the Indicator Bacterial Pathogens following Agar-well Diffusion Method



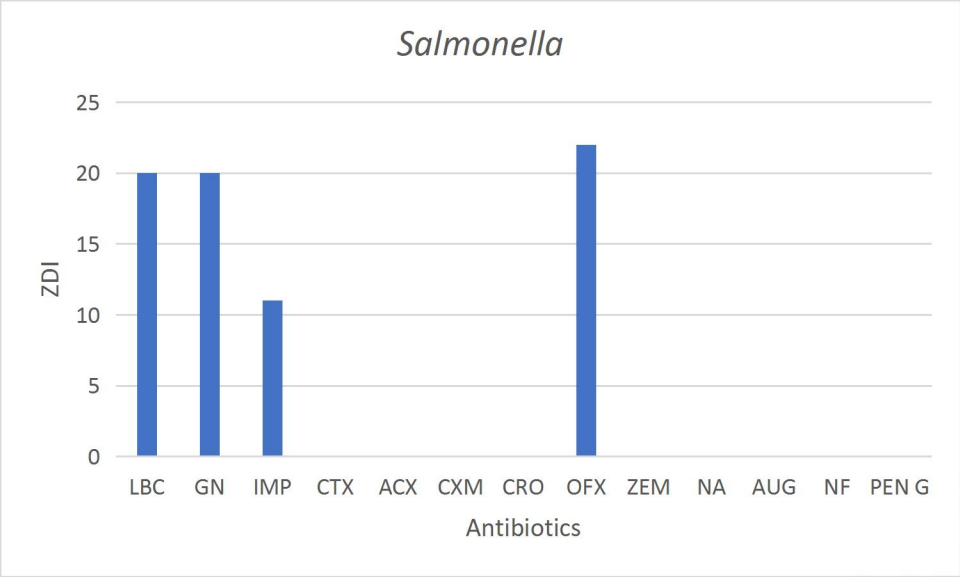
Antibacterial Activity of LAB Isolates (NG2) and Penicillin against the Indicator Bacterial Pathogens following Agar-well Diffusion Method



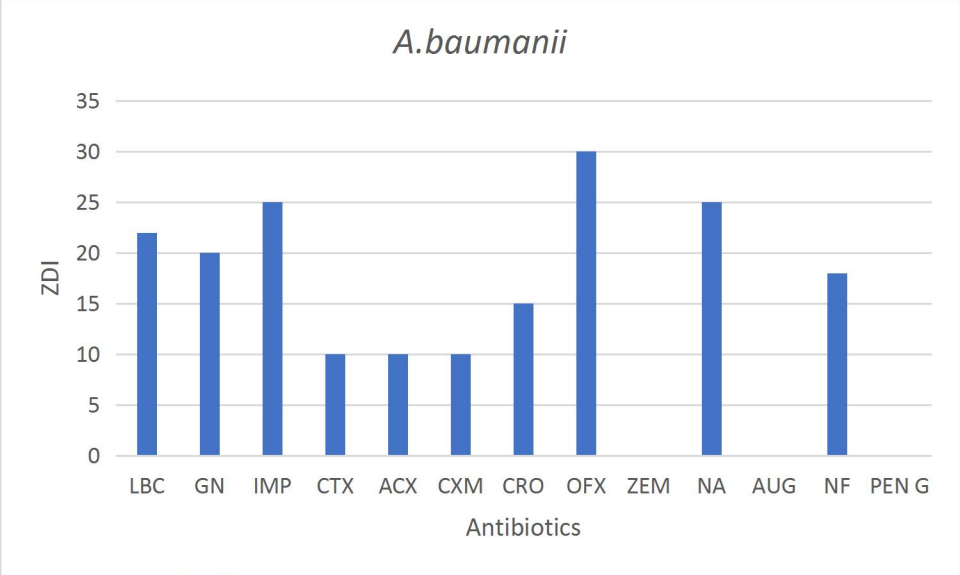
Antibacterial Susceptibility of Indicator Bacterial Pathogens



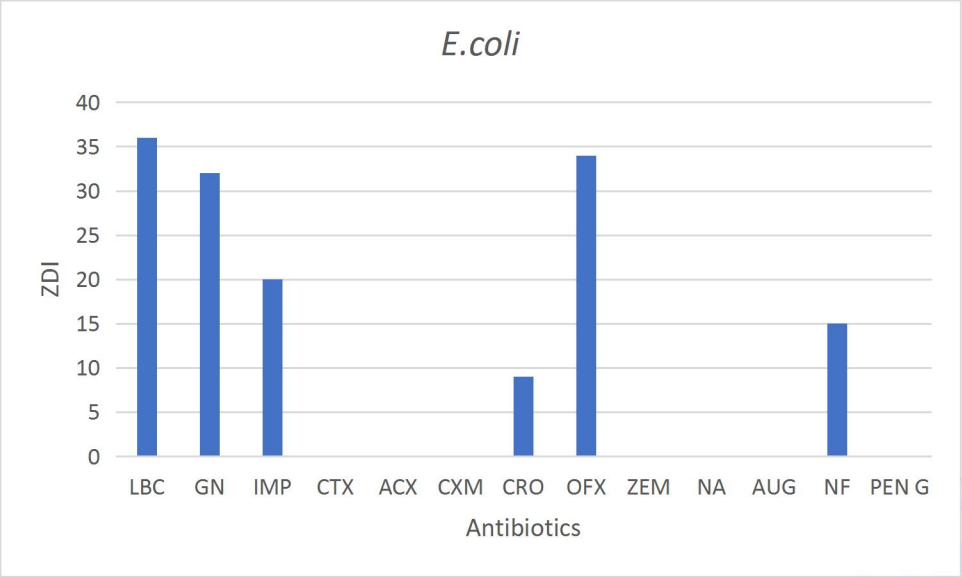
Antibacterial Susceptibility of Indicator Bacterial Pathogens



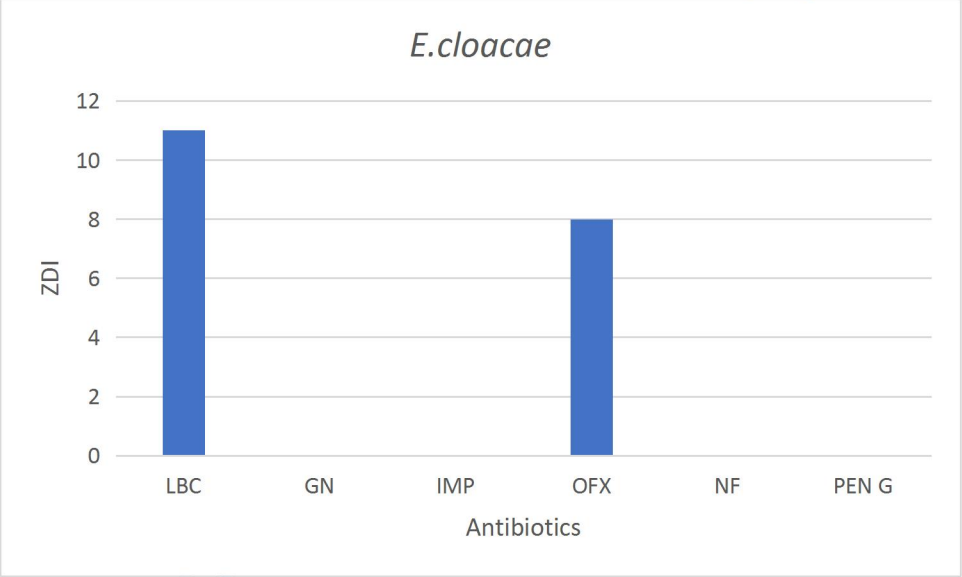
Antibacterial Susceptibility of Indicator Bacterial Pathogens



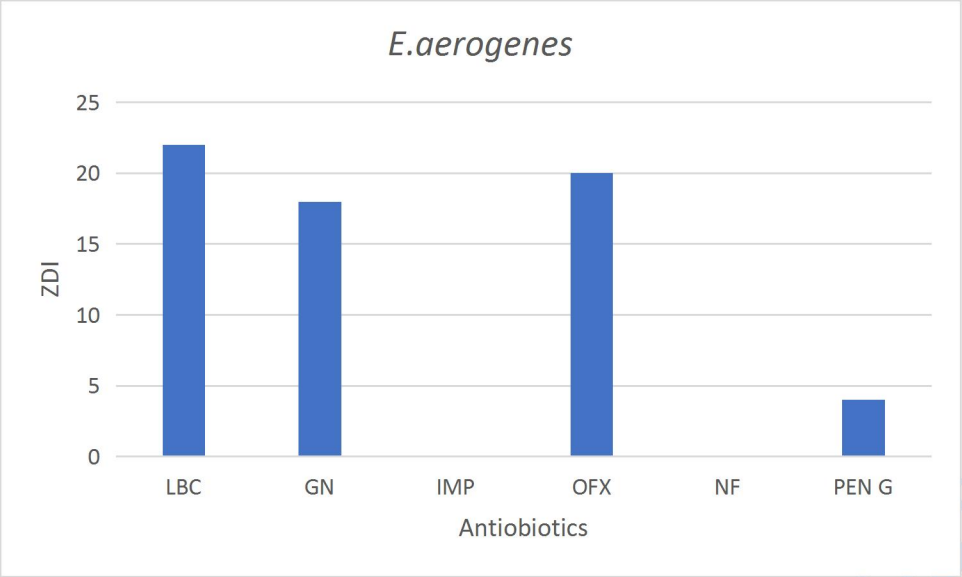
Antibacterial Susceptibility of Indicator Bacterial Pathogens



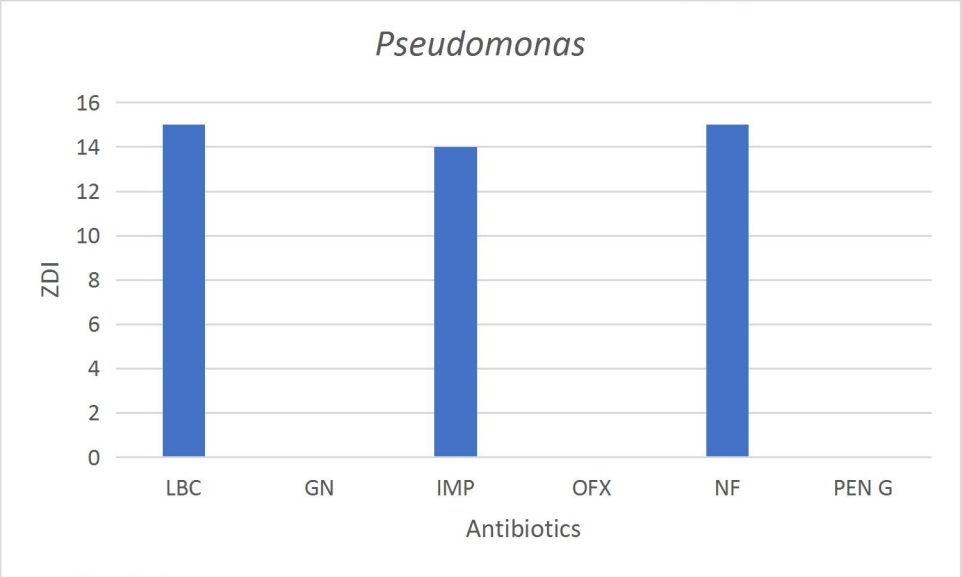
Antibacterial Susceptibility of Indicator Bacterial Pathogens



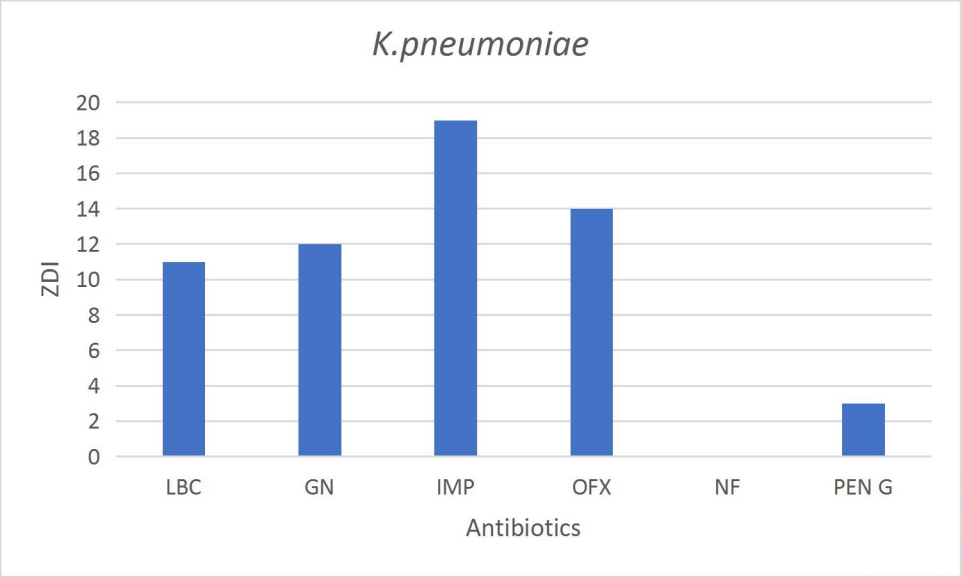
Antibacterial Susceptibility of Indicator Bacterial Pathogens



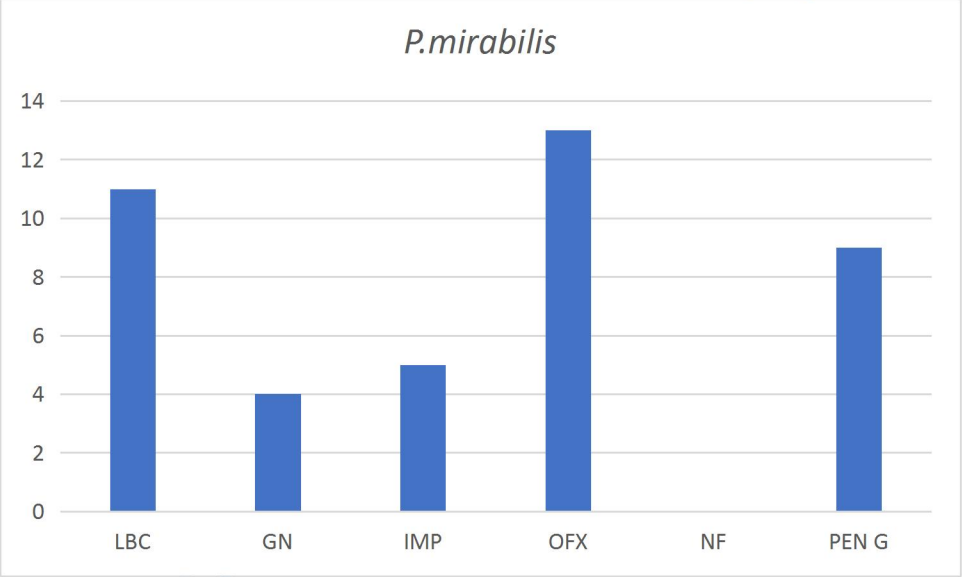
Antibacterial Susceptibility of Indicator Bacterial Pathogens



Antibacterial Susceptibility of Indicator Bacterial Pathogens



Antibacterial Susceptibility of Indicator Bacterial Pathogens



Antibacterial Susceptibility of Indicator Bacterial Pathogens

Bio-data

A. Personal Data:

1.	**Full Name:**	Ifeoluwa, Omodesola ODUNUGA No. 6, Orire Street, Soka, Ibadan, Oyo State. ifeodunuga24@gmail.com 08104085949
2.	Date and Place of Birth:	27th June, 1998; Abeokuta
3.	Nationality:	Nigerian
4.	State of Origin	Ogun State
5.	Name and Address of Next of Kin:	Dr J.B, Odunuga 27, Oloruntedo Street, Obantoko, Abeokuta, Ogun State

B. Educational Background:

Educational Institution Attended with Dates and Qualification:

School Attended	**Dates**	**Qualifications**
❖ Foursquare International School	.2002-2008	First Leaving Sch. Cert.
❖ Homaj Group of Schools, Ondo.	2008-2014	West African Exam. Cert.
❖ Bowen University	2014-2018	B.Sc. Microbiology
❖ Lead City University, Ibadan	2021-2023	M Sc Medical Microbiology in view

C. Working Experience with Dates:

❖ Business Development Specialist, Future Builders LLC	2022 - 2023
❖ Business Development Specialist, Data2Bots	Aug 2023 till Date

D. Publication

- ❖ Odunuga I.O, Adesina. F*; Potential of Bacteriocin and Antibiotics as Synergestic Therapeutic Agent against Resistant Strains of Bacteria Associated with Skin Infections.* 2023.

E. Academic Membership

American Society for Microbiology

Microbiology Professionals

F. Major Conference Attended with Dates

MedLab West African Conference	September, 2023
Medic West African Conference	September, 2023

G. Referees

Professor A.O Obadina

Professor K.E Oyadiran

Signature **Date**

The University Compliance Certification

This is to certify that, this Thesis written by **Ifeoluwa, Omodesola Odunuga** with Matric No**. LCU/PG/002352,** in the Department of Biological Science, Faculty of Natural and Applied Sciences, Lead City University, Ibadan is in full compliance with the approved University format and style.

_____ _____

Signature **Date**