Drug resistance in respiratory infections and Lysin as a Potential Therapeutics: A review

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of
Bachelor of Science in Microbiology

Mathematics and Natural Sciences
Brac University
July 2022

Declaration

It is hereby declared that

1. The thesis submitted is our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. We have acknowledged all main sources of help.

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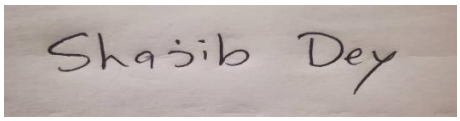

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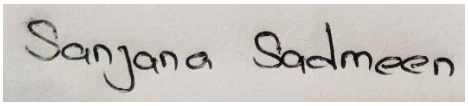

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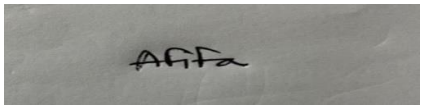

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Ethics Statement

Hereby, we consciously assure that for the manuscript 'A review on Drug Resistance of Respiratory Infections and Lysin as Potential Therapeutics' the following is fulfilled:

- This material is the authors' own original work, which has not been previously published elsewhere.
- The paper is not currently being considered for publication elsewhere.
- The paper reflects the authors' own research and analysis in a truthful and complete manner.
- The paper properly credits the meaningful contributions of co-authors and co-researchers.
- The results are appropriately placed in the context of prior and existing research.
- All sources used are properly disclosed (correct citation). Literally copying of text must be indicated as such by using quotation marks and giving proper reference.
- All authors have been personally and actively involved in substantial work leading to the paper and will take public responsibility for its content.

The violation of the Ethical Statement rules may result in severe consequences. We all the authors agree with the above statements.

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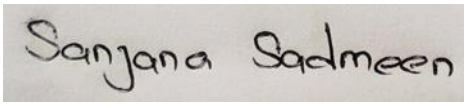

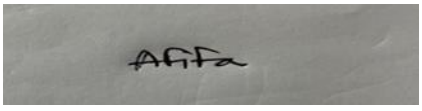

Abstract

The emergence and spread of respiratory drug-resistant bacteria that have gained novel resistance mechanisms, resulting in antimicrobial resistance, continues to pose a danger to our capacity to treat common respiratory infections. The primary objective of this paper's findings is to address this major problem. Bacteriophage-encoded lytic enzymes have long been studied as a potential alternative to antibiotics in the fight against bacterial infections. These enzymes, which function by degrading peptidoglycan, a crucial part of the bacterial cell wall, have an antibacterial effect. Multiple studies have previously shown that using different lysins to counteract various pathogenic bacteria that cause respiratory tract infections has had positive outcomes. High-dose Cpl-1 eliminates *Streptococcus pneumoniae* faster than vancomycin and stimulates cytokine production. Lysin 23TH 48 is effective against *Streptococcus pneumoniae*. LysP108's unique amino acid sequence and domain structure may be combined with drugs to prevent bacterial antibiotic resistance. *Streptococcus pyogenes* cells could be destroyed by PlyC, a unique multimeric enzyme that is effective against group *A streptococci*. Art-175 is a thermostable artilysin produced by mixing lysin KZ144 with sheep myeloid AMP-29 (SMAP-29). Art-175 suppressed persister development, a post-antibiotic bacterial subpopulation. LysCA and LysG24 may reduce pulmonary inflammation and LPKP growth. Clinical symptoms and bacterial load in the mouse lungs favored LysCA. LysAB3, LysAB4, PlyAB1, and LysABP-01 were designed to kill *Acitenobacter baumannii*. PlyF307 may kill planktonic and biofilm *Acitenobacter baumannii* isolates, including MDR strains. This review study addressed the significant antibiotic resistance of respiratory pathogens that are no longer effectively treated by antibiotics and demonstrated an alternative, the use of lysin, based on several successful in vivo and in vitro studies.

Keywords: Respiratory infections; drug-resistant; potential alternative; bacteriophage-encoded lytic enzymes; peptidoglycan; lysin.

Dedication

To Our Family &
Ourselves

Acknowledgement

We acknowledge the gracious support of the chairperson, Professor A F M Yusuf Haider, Ph.D., and the program coordinator for the microbiology department, Mahbubul Hasan Siddiqee, Ph.D. We would like to express our sincere appreciation and debt of gratitude to our thesis advisor Assistant Professor Fahim Kabir Monjurul Haque, Ph.D., for his guidance, inspiration, kind assistance, and unwavering enthusiasm at every stage of this study and every stage of our education at this university. Working with him has been a true pleasure for us.

We really appreciate Brac University's Department of Mathematics and Natural Sciences for giving us the chance and resources we needed to finish our thesis.

We would especially want to thank our family for their support, without which we would not have been able to complete this thesis.

Drug resistance in respiratory infections and Lysin as a Potential Therapeutics: A review

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List of Acronyms

URTI - Upper respiratory tract

LRTI - Lower respiratory tract

GBD - Global Burden of Disease Study

CDC - Centers for Disease Control and Prevention

HGT - Horizontal gene transfer

PG - Peptidoglycan

VISA - Vancomycin-intermediate *Staphylococcus aureus*

TB - Tuberculosis

CW - Cell wall

CD - Cell domain

CWBD - Cell wall binding domain

SEM - Scanning electron microscope

EDTA - Ethylenediaminetetraacetic Acid

HHP - High hydrostatic pressure

OM - Outer membrane

AMP - Antimicrobial peptide

GI - Gastrointestinal tract

UTI - Urinary tract infection

LPKP - Lung pathogenic Klebsiella pneumoniae

MDR - Multi drug resistance

G+ - Gram positive

G- - Gram negative

OMP - Outer membrane proteins

PNSP - Penicillin-Resistant Pneumococci

OECD - Organization for Economic Co-operation and Development

AMR - Antimicrobial resistance

ARMs - Antimicrobial-resistant microorganisms

G7 - Group of seven

AMTs - Antimicrobial therapies

CLSI - Clinical and Laboratory Standards Institute

ANSORP - Asian Network for Surveillance of Resistant Pathogens

MRSA - Methicillin-resistant Staphylococcus aureus

HA - Health care-associated

CA - Community-Associated

HAP - Hospital-acquired pneumonia

Chapter 1 Respiratory Bacterial Infections

Infections of the respiratory tract (RTIs) are among the most prevalent and critical infections in clinical medicine, posing both therapeutic and economic challenges. Bacteria of both gram-positive and gram-negative types can infect the respiratory tract. Despite the fact that the illnesses they cause might range from minor to severe, the bacteria are usually isolated to the respiratory system. Organisms infect the respiratory system by inhaling droplets and penetrating the mucosa, causing respiratory infections. The presence of colonizing bacteria triggers an immunological and inflammatory response in the host, including immune system cells (Cole & Wilson, 1989). Bacteria that are able to evade the mucociliary defense system and enter the lungs might potentially continue to colonize the organ. The features of the bacterial species and the epithelial cells that line the respiratory tract are the primary factors that determine whether or not bacteria are able to colonize and infect the respiratory system. Redness, edema, bleeding, and exudate are all possible side effects of epithelial damage (Baron S., 1996). Pathogenic bacteria attach to the mucous membranes, where they usually colonize (Cappelletty, 1998). Following influenza infections, bacterial respiratory infections are commonly recognized as a cause of mortality in humans, and numerous mechanisms have been identified by which initial viral respiratory infections enhance susceptibility to subsequent bacterial infections (Hodgson et al., 2012). Whenever bacterial elimination is prolonged, time for bacterial-mucosal binding might occur (Niederman, 1990). The density of cilia, the amount of mucins on the cell surface, and the presence of bacterial surface antigens all play a role in determining whether or not bacteria are able to attach themselves to an epithelial cell (Johnson, 1995) (Wanner, 1977). Bacteria are capable to produce structures on their surfaces, such as pili, that assist them in adhering to the surface of epithelial cells, which is necessary for their survival. Pili is produced by H. influenzae, and they may be found adhered to human nasopharyngeal epithelial cells. (Disease, 1992), (Apicella et al., 1984) (Van Alphen et al., 1988) (Bakaletz et al., 1988).

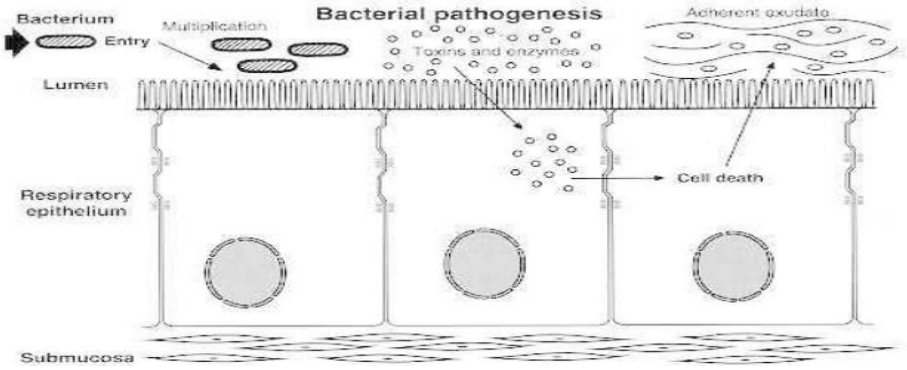

Figure 1: Pathogenesis bacterial mucosal respiratory infections (Baron S., 1996)

There are two types of infections that may affect the respiratory system The first type involves infections in the upper respiratory system, whereas the second type involves illnesses in the lower respiratory system. Infections of the upper respiratory tract and infections of the lower respiratory tract may be differentiated from one another based on the part of the respiratory system that is affected. Upper respiratory tract infections affect the structures in or above the larynx, whereas lower respiratory tract infections affect the airways below the larynx. Children, adults, and those with immune system abnormalities are most vulnerable to these diseases. People with heart disease or other lung problems are more likely to get acute respiratory infections. Children often get sick as a result of acute respiratory infections, the majority of which are caused by infections of the upper respiratory tract. Bacterial pathogens cause severe lower respiratory infections, but upper respiratory infections cause most acute respiratory infections. (Kemper Alston & Fahrner, 2003). The upper respiratory tract is habitat to a vast range of commensals and potential pathogens, including *Haemophilus influenzae*, *Staphylococcus aureus*, *Streptococcus pneumonia* and *Moraxella catarrhalis* all of which can become pathogens and cause infectious diseases (Watson et al., 2006). In some situations, they can move to other places. They can cause infections of the upper respiratory tract like otitis media, pharyngitis, tonsillar hypertrophy, sinusitis, and others. They can also cause infections of the lower respiratory tract like pneumonia, bronchitis, bronchiolitis, and invasive infections like meningitis, pneumonia with bacteremia, and sepsis.

1.1 Upper respiratory tract infection

Upper respiratory tract infections are most frequent in clinical sectors. As a consequence of health issues such as smoking, allergies, and airborne environmental contaminants, the upper respiratory tract may become susceptible to bacterial infection.

A pathogen infiltrating the upper airway mucosa is the most common cause of a URTI. The organism is usually obtained by inhalation of infected droplets. The sinuses, nasal passages, pharynx, and larynx are all part of the upper respiratory system. These structures transport the air we breathe from the outside to the trachea and then to the lungs, where we breathe (Jerry et al., 2022). The cilia that capture infections, mucus capturing organisms, the angle between the pharynx and nose, and ciliated cells in the lower airways that convey pathogens back to the pharynx, are all barriers that prevent pathogens from adhering to the mucosa (Thomas M. & Bomar PA., 2021). Pathogens (viruses and bacteria) must overcome many physical and immunologic barriers in order to infiltrate the mucus membrane of the upper airways. Different pathogens have varying abilities to overcome the body's defense system and cause infections (Jerry et al., 2022). Due to the widespread usage of antibiotics, bacterial resistance has developed and spread. In upper respiratory tract infections, the major mechanisms of bacterial resistance to antimicrobials include enzymatic inhibition, membrane impermeability, alteration of the ribosomal target, modification of target enzymes, and active pumping out of antibiotics (Cappelletty, 1998).

Nasopharyngitis often affects the nasal passages, pharynx, hypopharynx, uvula, and tonsils. Rhinitis affects the nasal mucosa, while rhinosinusitis involves the nose and paranasal sinuses. Group A and Group C beta-hemolytic streptococci are the two most common bacteria that cause upper respiratory infections. Streptococcal pharyngitis, known as strep throat, is a common upper respiratory illness caused by *Streptococcus pyogenes. Streptococcus pyogenes*, on the other hand, is the sole organism that necessitates an etiologic diagnosis and treatment. Various study data shows that acute pharyngitis is a primary source of improper antibiotic usage in clinical practice (Nakhoul & Hickner, 2013; Anjos et al., 2014). Acute bacterial maxillary sinusitis is a common illness acquired in the community. The epiglottis can get infected with bacteria such as *Haemophilus influenzae*, causing it to enlarge and potentially restrict the airway. This causes severe breathing difficulties and can be life-threatening (Baiu & Melendez, 2019). Haemophilus influenza type B is the most common bacterial cause of epiglottitis in adults (Fontanarosa et al., 1989). With the exception of influenza, which is caused by a virus, complications from upper respiratory tract infections are rare. Secondary bacterial pneumonia, sinusitis, otitis media, bacterial coinfection, and the progression of preexisting conditions, such as asthma and chronic obstructive pulmonary disease, are all possible complications of influenza infection. Pneumonia is one of the most severe influenza complications in children and a primary cause of morbidity and death (Thomas & Bomar, 2021).

1.2 Lower respiratory tract infection

Infections of the lower respiratory tract affect the airways below the larynx. Lower respiratory tract infections (LRTIs) are the fourth leading cause of mortality, according to the Global Burden of Disease 2015 research (Wang et al., 2016). Lower respiratory infections, in general, remain longer, are more severe, and are among the most prevalent infectious illnesses with potentially fatal consequences (Khan et al., 2015). Though viruses cause the majority of LRTIs, but antibiotics are frequently provided for their treatment without any laboratory testing, which can contribute to the evolution of antimicrobial resistance.

Acute bronchitis, pneumonia, acute exacerbations of chronic obstructive pulmonary disease/chronic bronchitis (AECB), and acute exacerbations of bronchiectasis are all examples of lower respiratory tract infection (LRTI). A bacterial pathogen was detected in 19% to 43% of patients in the few trials where possible pathogens were adequately isolated from primary care patients with LRTI (Macfarlane et al., 2001) (Graffelman et al., 2004) (Hopstaken et al., 2005). (A. et al., 2007). Children under five are especially susceptible to LRTIs, which are the leading cause of mortality in this age range (Wang et al., 2016). According to the Global Burden of Disease 2015 study, pneumococcal pneumonia is the most widespread cause of pneumonia, accounting for about 1.5 million (95% confidence range of 0.95–2.18 million) early deaths globally in 2015 (Wang et al., 2016). *Streptococcus pneumoniae* is the most frequent bacteria that causes pneumonia in humans. Community-acquired pneumonia is caused by distinct microorganisms in the community, but hospital-acquired pneumonia is caused by germs in the hospital. The two most prevalent acute LRTIs in clinical settings are acute exacerbation of chronic bronchitis (AECB) and community-acquired pneumonia (CAP) (Brar & Niederman, 2011). There are a number of different bacterial infections that may cause pneumonia, but some of the more frequent ones include *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Haemophilus influenzae*. On the other hand, bacteria such as *Mycoplasma pneumoniae*, *Legionella pneumophila*, *Chlamydophila pneumoniae*, and *Chlamydia psittaci* are the causes behind unusual cases of community-acquired pneumonia (CAP) (Prasad, 2012; Cunha, 2006).

The prevalence of community-acquired bacterial pneumonia (CABP) has changed as a direct result of the rise in antimicrobial resistance, which is a significant public health problem on a global scale (Jain et al., 2015; Musher et al., 2014; Ramirez et al., 2017). Bacteria are found in 1% to 10% of cases of acute bronchitis, according to statistical data (Clark et al., 2014; Gencay et al., 2010; Macfarlane et al., 2001). Acute bronchitis may be caused by a number of common bacteria, including *Chlamydophila pneumoniae*, *Mycoplasma pneumoniae*, and *Bordetella pertussis*, among others (Kinkade & Long, 2016). Inflammation, an increase in the amount of mucus that is produced, and a reduction in the function of the mucociliary system are all symptoms that may occur as a result of the pathogen proliferating in or on the epithelium. Several lung activities could also be affected. Epithelial swelling and necrosis may cause narrow airways to become obstructed, which can result in respiratory arrest in severe cases of bronchiolitis (Dasaraju & Liu, 1996).

Chapter 2 Loss of Antibiotic Effectiveness

Antibiotics are becoming less effective against bacteria. Antibiotic resistance has been considered one of the most significant worldwide risks of the twenty-first century (Conly & Johnston, 2005). It has been shown that inadequate use and misuse of antibiotics prescribed for the treatment of respiratory tract infections is one of the key factors to the development of bacterial resistance (Gonzales et al., 1997). The existence of mobile genes on plasmids that travel and transfer among bacterial populations and the production of biofilms during the quorum-sensing-regulated process that releases beta-lactamase, which is responsible for the breakdown of many antibiotics, these are also some major other contributing factors for antibiotic resistance in bacteria (Bennett PM, 2009; Wilke et al., 2005). An alarmingly high percentage of bacteria throughout the globe have developed resistance to the antibiotics that are now available, and a large portion of the mortality rate in respiratory disorders may be related to acute infections caused by a wide variety of bacterial species. Antimicrobial resistance is a developing public health risk directly related to antibiotic use (Raft et al., 2017). Excessive antibiotic use has resulted in bacterial resistance to antibiotics, posing a serious public health concern, especially given the diminishing availability of newer medicines (Arason et al., 1996).

When a bacterium is antibiotic-resistant, it may multiply or survive during the antibiotic treatment that would typically inhibit or kill organisms of the same species. Microorganisms may be resistant to an antibiotic from the start, or they can acquire resistance after exposure. Though antibiotic resistance is an unavoidable result of antibiotic usage, uncontrolled use of antibiotics plays a key role in the spread of resistance throughout the world. In the United States, for example, far too many antibiotics are administered needlessly to individuals. Each year, the CDC estimates that 47 million antibiotic courses are administered in U.S. doctors' offices and emergency rooms for diseases that don't require antibiotics, such as colds and the flu. This corresponds to around 28% of all antibiotics recommended in these situations. Approximately 25,000 individuals die each year in the European Union as a result of an illness caused by bacteria that are resistant to several antibiotics, with this number expected to rise to 390,000 by 2050 if current trends continue (Graham, 2017). In many areas, antibiotics are accessible "over the counter" or over the internet, giving non-prescribers full and direct access to these medications. Following the emergence of resistance, the selection pressure exerted by continued antibiotic use, failure to adhere to infection control measures, and poor hygiene (particularly in terms of hand hygiene, sanitary conditions, and food preparation), which can occur within and without healthcare settings, facilitates the subsequent dissemination of resistant pathogens. Antibiotic resistance costs society a lot of money in terms of higher mortality, morbidity, healthcare expenditures, and lost time at work. Antibiotic resistance has a significant financial impact, but there is also significant concern about the absence of new antibiotic research. In the previous two decades, just two new antibiotic classes have been developed and introduced into clinical practice. These include oxyazolidinones like linezolid and lipopeptides such as daptomycin. Rather than being new classes of antibiotics, many of the newer antibiotics are variations of previous medicines.

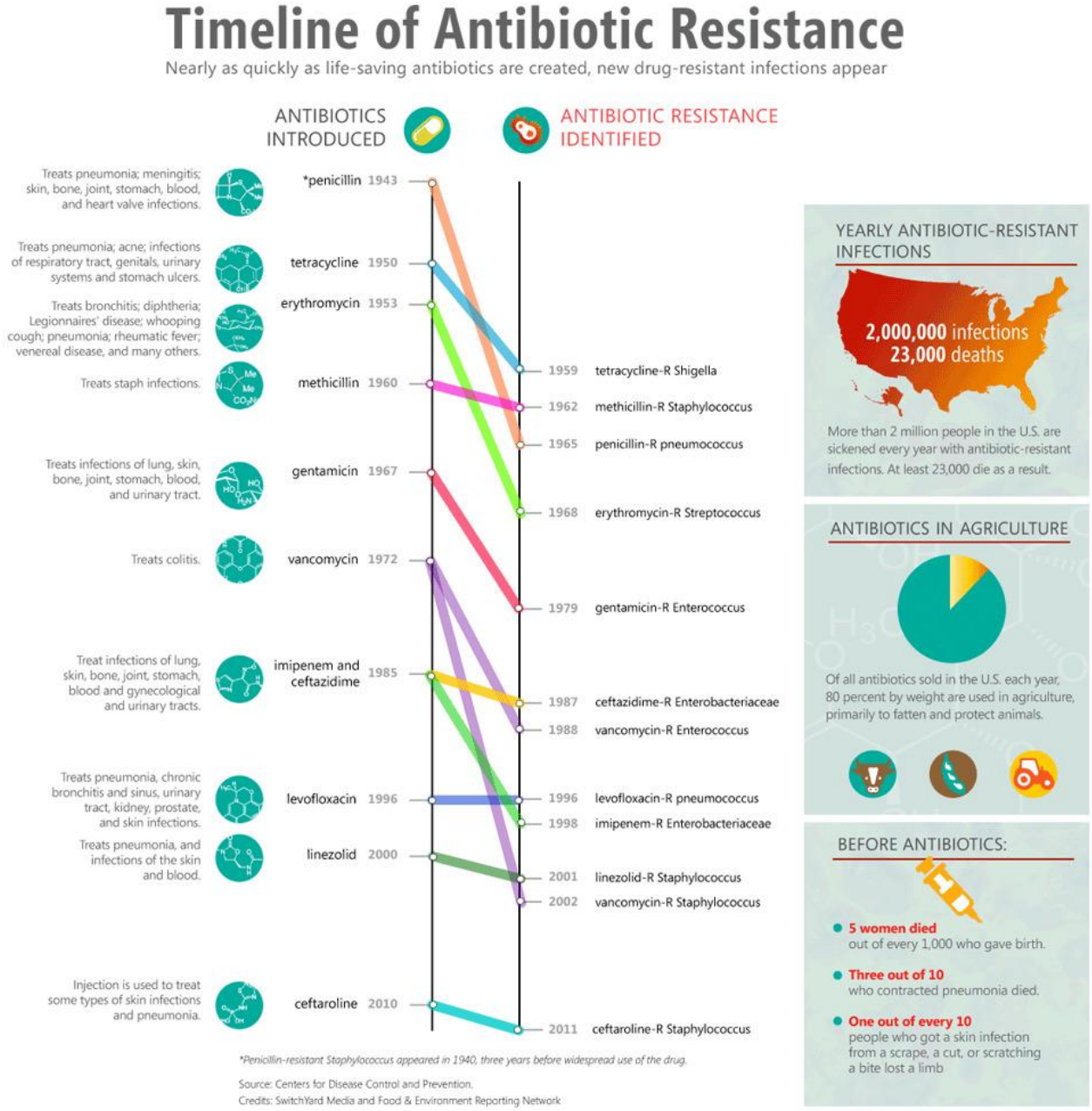

Figure 2: Infographic depicting a timeline of antibiotic resistance: when antibiotics were introduced and when their resistance was first identified. (Graham, 2017)

2.1 Current trend in Resistance

AMR is the root of serious negative effects on people's health in both developed and developing nations. A very small number of agents are responsible for the vast majority of the health burden. Nine agents are included in the WHO report on the worldwide status of ARMs (WHO, 2014) as being primarily responsible for this burden (table 1). At least five of these nine agents are very common in every G7 and OECD nation. In hospitals, infections caused by *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* are widespread and often affect susceptible people (e.g., neonates). The most common cause of community-acquired pneumonia, one of the primary causes of death in young children, is *Streptococcus pneumoniae*. Although *Staphylococcus aureus* is widespread in the general population, it is the most prevalent cause of post-operative infections and certain strains may result in toxic shocks.

Although it is less prevalent in G7 nations, drug-resistant tuberculosis (TB) represents a persistent threat. Globally, 8.7 million individuals contracted TB in 2012, and the illness claimed the lives of 1.3 million people (WHO, 2014). In the same year, it was estimated that there were 450,000 instances of multidrug-resistant tuberculosis (MDR-TB), or about 3.6 percent of all new cases and 20.2 percent of patients that had already been treated for the disease. The anticipated number of people with TB worldwide in 2013 was around 122,000, which is merely a small portion of the frequent cases in G7 nations. Two aspects, nevertheless, should prevent complacency. Initially, rising incidence rates were first seen in several G7 nations between 2005 and 2010, after years of declining trends. Moreover, rates of MDR-TB are similar to the global average among G7 nations. For both newly diagnosed and previously treated cases of TB, the prevalence of MDR-TB is 3.6% and 20.2 percent% globally, respectively. According to the G7, the prevalence of MDR-TB varies between 0.5 and 2.6 percent for newly diagnosed patients and between 1.6 and 21.0% for individuals who have already received treatment.

Antibiotic-resistant bacteria (table 1) raise the community health burden in many ways as compared to illnesses that are susceptible to AMTs. The first effect of ARMs is to prolong morbidity and lengthen the duration of the infectious illness. Second, ARMs raise the risk of acquiring additional problems, or comorbidities. Third, patients are more prone to having negative responses or unintended side effects since more rigorous treatments are needed. Last but not least, individuals with ARM infections had greater fatality rates.

Infectious agent	Common infection sites or clinical conditions	Common resistances	Average resistance rates throughout the G7 nations [min-max]
Escherichia coli	bloodstream, skin, soft tissues, meningitis in newborns, the urinary system, the blood, the intra-abdominal space, and foodborne illnesses	3rd generation cephalosporins; carbapenem; fluoroquinolones,	3rd generation cephalosporins: 12.1% [8.0%-19.8%] fluoroquinolones: 27.7% [17.5%-40.5%]
Klebsiella pneumoniae	urinary tract, bloodstream, meningitis in neonates	3rd generation cephalosporins, carbapenem, cotrimoxazole; fluoroquinolones	3rd generation cephalosporins: 17.3% [4.0%-45.9%] carbapenem: 5.5% [0.0% - 26.7%]
Staphylococcus aureus	infections in the bloodstream, skin, soft tissue, bone, surgical wounds, toxic shock syndrome, and foodborne illness	methicillin	30.5% [13.6% - 53.0%]
Streptococcus pneumoniae	bloodstream infections, pneumonia, meningitis, and otitis media	penicillin	8.3% [0.1% - 42.2%]
Mycobacterium tuberculosis	tuberculosis	Rifampicin, streptomycin, fluoroquinolone, isoniazid	Retreatments: 9.1% [1.6%-21.0%] New cases: 1.3% [0.5% - 2.6%]

Table 1: Resistant rates of antibacterial resistant pathogens and their infections sites (Antimicrobial Resistance - OECD, 2015)

It is possible for the circumstances surrounding antimicrobial resistance to differ from country to country or region to region. On the other hand, Asia is generally regarded as the world 's leading region in terms of antimicrobial resistance. The percentage of major bacterial pathogens that are resistant to antimicrobial treatment has been steadily climbing over the past few decades (Jean & Hsueh, 2011; Nickerson et al., 2009). In Asia, the mortality rate of β-lactam and macrolide resistance in *Streptococcus pneumoniae* have been found to be quite high, according to published research (Kim et al., 2012; Song et al., 2004). Specifically, erythromycin tolerance has dramatically increased in a number of Asian countries, where it was found that over 70% of clinical isolates were completely resistant to the drug (Kim et al., 2012; Song et al., 2004). The prevalence rates of penicillin resistance were 0.7% and 57.5% in nonmeningeal and meningeal isolates, respectively, based on the revised CLSI breakpoints for parenteral penicillin (resistant 8 g/ml for nonmeningeal isolates and 0.12 g/ml for meningeal isolates) (Kim et al., 2012). This information was obtained from a recent perspective surveillance study that was carried out by the Asian Network for Surveillance. When earlier penicillin susceptibility breakpoints were used, new data indicated a consistently high incidence of penicillin nonsusceptibility in Asian nations (Kim et al., 2012; Song et al., 2004; Song et al., 1999). This is in contrast to previous ANSORP investigations, which found a lower prevalence of penicillin nonsusceptibility.

However, according to the revised CLSI breakpoints, only China (2.2%) and South Korea (0.3%) had completely resistant isolates, while the prevalence rate of PNSP in nonmeningeal isolates was only 4.6%. The greatest rates of erythromycin resistance were found in China (96.4%), Taiwan (84.9%), and Vietnam. Overall, the area had a relatively high prevalence of erythromycin resistance (72,7%) (80.7%). MDR was identified in 59.3% of the isolated strains from Asian countries. The percentage of *Streptococcus pneumoniae* infections that were resistant to the antibiotic levofloxacin rose dramatically in a hospital in Taiwan between the years 2001 and 2007, from 1.2 % to 4.2% (Hsieh et al., 2010). The highest levels of levofloxacin resistance were found in isolates that were first found in Taiwan (at a rate of 6.5%) and South Korea (at a rate of 4.6%) respectively (Kim et al., 2012). Korea reported a case of bacteremic pneumonia caused by an extremely drug-resistant strain of *Streptococcus pneumoniae*. (Kang et al., 2012). Except for vancomycin and linezolid, this variant of *Streptococcus pneumoniae* was resistant to at least one drug in all classes of antibiotics. In Asia, MRSA is the major cause (> 50%) of healthcare infections such as pneumonia, surgical site infections, and sepsis. Since MRSA is responsible for the deaths of more than 19,000 people per year in the United States alone, it is possible that many Asian nations will experience a very high number of fatalities as a result of this infection (Jean & Hsueh, 2011; Grundmann & Hellriegel, 2006). According to the findings of the ANSORP research, the prevalence of MRSA among HA *Staphylococcus aureus* infections was comparatively significantly low (22.6%), as well as in the Philippines (38.1%). On the other side, countries with extraordinarily high MRSA rates included Vietnam (74.1%), Korea (77.6%), and Sri Lanka (86.5%). Community-associated MRSA infections have increased globally in recently (Vandenesch et al., 2003; Naimi et al., 2003). According to the ANSORP research, MRSA prevalence in CA *Staphylococcus aureus* infections differed by nation: Sri Lanka had an MRSA prevalence of 38.8%, Taiwan had a 34.8%, the Philippines had a 30.1%, Vietnam had a 30.1%, Korea had a 15.6%, Hong Kong had an 8.5%, India had a 4.3%, and Thailand had a 2.5% (Song et al., 2011). Carbapenem-resistant *Pseudomonas aeruginosa* is relatively common in the countries

of Asia, and multidrug-resistant non-fermenting bacteria are found all across the region (Lee et al., 2009; Lee et al., 2005). In the ANSORP analysis on HAP, *Pseudomonas aeruginosa* had resistance to imipenem, ceftazidime, cefepime, piperacillin-tazobactam, and ciprofloxacin at rates of 36.9%, 34.7%, 27.7%, 27.2%, and 30.1%, respectively. China has the world's highest incidence of *Pseudomonas aeruginosa* strains resistant to the antibiotic imipenem (56.9%). The mortality rate of resistance to imipenem in HAP associated with Acinetobacter spp. was quite strong in Asian countries, at 67.3 %; it was especially high in Malaysia (86.7 %), Thailand (81.4%), India (85.7%), and China (58.9%) (Kim et al., 2013). Acinetobacter spp. had rates of 82.0 and 51.1% for both multidrug resistance and strong multidrug resistance. In China, Taiwan, and Korea, the percentage of *Acinetobacter baumannii* strains resistant to the antibiotic imipenem has steadily increased recently (Lee et al., 2011; Jean et al., 2009; Wang et al., 2010; Kuo et al., 2012).

2.2 Limitations of Antibiotics

Antibiotics are a potent type of therapy that fights bacterial infections and reduces mortality from numerous infectious illnesses that are causing epidemics. They have the ability to kill or limit bacterial development in the body, as well as treat or fight bacterial infections (Soffar, 2021). Antibiotics are extremely effective in combating disease, but they can also have negative side effects (Everett, 2021). Antibiotics have the potential to harm beneficial microorganisms in the body. The longer an antibiotic is taken, the greater the potential for harm to the immune system. Antibiotics can cause a variety of adverse effects, ranging from stomach problems to bone damage to sun sensitivity (Antibiotics, 2022). As the nature of the microbial species in our intestine changes, many antibiotics cause diarrhea. It may increase the quantity of pathogenic bacteria that cause diarrhea, such as *Clostridium difficile*. Some medications, such as sulfa, which is contained in many antibiotics, might induce bad effects based on our drug allergies. Any medicine, including antibiotics, can cause allergic reactions (Soffar, 2021).

Antibiotics have been linked to an elevated threat of cardiovascular incidents and death in a randomized therapeutic trial and multiple observational research. Furthermore, other non-Cochrane Reviews, which are already outdated, have evaluated the effects of antibiotics on coronary heart disease and found mixed results (Illinois, 2019). Although seizures are uncommon as a result of antibiotic side effects, antibiotics such as ciprofloxacin, imipenem, and cephalosporins such as cefixime and cephalexin are more likely to provoke seizures. Tendonitis and tendon rupture have been linked to antibiotics like ciprofloxacin. Certain antibiotics might cause cardiac issues such as an irregular pulse or low blood pressure in rare circumstances. Sulfamethoxazole and beta-lactam antibiotics are more likely to cause leukopenia and thrombocytopenia. Antibiotics reduce the number of lactobacilli, a beneficial bacterium found in the vaginal area. This "healthy bacteria" aids in the control of Candida, a naturally occurring fungus. A yeast infection can emerge when this natural equilibrium is shifted in favor of Candida growth. Light sensitivity is caused by antibiotics like tetracycline (Illinois, 2019). Antibiotic-resistant infections that need the use of second- and third-line medicines can damage patients by producing major side effects like organ failure and delaying care and recovery for months.

Resistance to antibiotics in disease-causing bacteria is rapidly becoming a major cause of morbidity, and it is one of the most significant threats to the survival of mankind in the last few

decades (Walker et al., 2011). Antibiotic usage adds to the rise of bacterial infections (Soffar, 2021). The most serious problem with antibiotics is overuse, which can cause bacteria to develop resistance towards the antibiotic being administered. If the bacterium develops a resistance, it might cause serious problems in the future if not handled appropriately. When existing difficult-to-treat germs have the appropriate mix of resistance mechanisms, all antibiotics become ineffective, resulting in untreatable diseases. Bacteria and fungi can contain genes for numerous forms of resistance. Antibiotic-resistant bacteria can share their resistance mechanisms with bacteria that haven't been exposed to antibiotics, which is concerning. It makes it less likely that infections may be properly treated and it increases the risk of morbidity and death associated with diseases that are often caused by bacteria (Cassir et al., 2014). Each year, more than 2.8 million instances of antibiotic-resistant bacterial infections occur, resulting in at least 35,000 fatalities (CDC, 2021).

There are three potential routes that might result in antibiotic resistance: change in drug-target interaction, antibiotic efflux from the cell, and direct destruction or modification of the molecule (Frieri et al., 2017) *Pseudomonas aeruginosa* bacteria may manufacture pumps to get rid of antibiotics such fluoroquinolones, beta-lactams, chloramphenicol, and trimethoprim. Carbapenemases are bacteria-produced enzymes that break down carbapenem antibiotics as well as most other beta-lactam antibiotics. The majority of *Escherichia coli* and *Klebsiella pneumoniae* isolates that have been reported by the European Antimicrobial Resistance Surveillance Network (EARS-Net) in 2012 were resistant to at least one of the antimicrobials that were tested, and many of these isolates had resistance to fluoroquinolones, aminoglycosides, and third-generation cephalosporins. In *Escherichia coli* bacteria, the mcr-1 gene can add a molecule to the cell wall's surface that inhibits the antibiotic colistin from attaching onto it. Certain *Staphylococcus aureus* germs are resistant to trimethoprim's effects (CDC, 2021). The cellular level of resistance and the community level of resistance are the two levels of antimicrobial resistance. The development of cellular resistance is aided by both intracellular gene mutations and Horizontal gene Transfer (HGT) of resistance genes from other bacteria. Also known as community level resistance, a population of bacteria may tolerate environmental stress that individual cells cannot. Antimicrobial resistance may increase as a result of this tolerance (Gerard, 2005).

Antibiotic treatment options are becoming more limited as the frequency of multidrug-resistant (MDR) bacterial infections causing hospital and community-acquired diseases rises (Cheng et al., 2016). Multidrug-resistant Gram-positive and Gram-negative bacteria are generally hard to fight, and in certain cases, standard medicines may not even be effective. There is now a scarcity of effective medications, effective prophylactic measures, and just a few new antibiotics, necessitating the creation of novel therapeutic options and antimicrobial treatments (Cassir et al., 2014). Various studies have shown that over the last 10 years, treatment resistance has significantly increased among community-acquired respiratory infections, particularly S. pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis (Felmingham et al., 2002). Despite the fact that antimicrobial therapy has been the standard of care for patients with bacterial infections, evidence on the influence of antibiotic therapy on respiratory disease outcomes is insufficient. Despite receiving proper antibiotic treatment, a significant percentage of high-risk individuals die. Even in the absence of antibiotic medication, few people die among low-risk patients (Metlay et

al., 2002). Standardized interpretations of drug levels of bacterial inhibition in vitro do not always correspond to actual drug levels in vivo (Craig et al., 1998).

Chapter 3 Lysin as Potential Alternative

Antibacterial treatment is among the most important healthcare breakthroughs the modern period, and it has since become one of the cornerstones of modern medicine, averting millions of deaths from bacterial illness. Antibiotics have drastically changed the outcomes of people with such illnesses, as well as the way we treat and cure diseases. Bacteria have a surprising variety of genetic pathways for antibacterial resistance, and there is a large pool of antibiotic resistance genes in nature that has developed over millions of years. The development and transmission of multidrug-resistant organisms has risen substantially during the last 50 years, according to organism and epidemiological data. Antibiotic resistance has a variety of negative consequences, the most serious of which is mortality. In individuals with bacteraemic pneumococcal pneumonia, infection with penicillin-resistant pneumococci, is associated with a fourfold increase in the risk of suppurative complications. Multidrug-resistant organisms have a harmful influence on those who aren't infected with them. Antibiotic resistance has a detrimental influence on all patients because it affects empiric antibiotic regimens, antibacterial classes that can be used, and the use of less effective medicines. This research focuses on a novel approach to combating growing multidrug resistance. Lysin might be a useful tool in the battle against multidrug resistance.

3.1 History of lysin

Lysins are among the most developed enzymes derived from the action of bacteriophages that break down the membrane of the bacterial cell, allowing progeny of phages to be released (Fischetti, 2008). They are peptidoglycan-hydrolyzing enzymes that are very efficient and selective, and they are produced as soluble cytoplasmic proteins (Jofre & Juan, 1999). Bacteriophages are obligatory parasites that must reproduce and grow in the presence of a bacterial host. They have two life cycles: lytic and lysogenic (Salmond et al., 2015). Phage have developed two methods for releasing their progeny from host bacterial cells over millennia. The filamentous phage is able to penetrate bacterial cell walls and escape without killing the target cells. (Russel et al., 1997), While non-filamentous phages utilize specialized lysins (encoded by single-stranded RNA or DNA phages) to either prevent the formation of peptidoglycans or hydrolyze the peptidoglycan (double stranded DNA phage encoded enzymes) (Borysowski et al., 2006). During the pre-antibiotic era, bacteriophages were discovered by d'Herelle in the year 1917 (d'Herelle, 1917), despite the fact that their antibacterial activity had previously been documented by Hankin in 1896 (Hankin, 1896) and Twort in 1915 (Twort, 1915) (Hermoso et al., 2007).

After phages were discovered, they were swiftly put to use against bacterial illnesses in animals and then in humans. A phage cocktail was administered to a child suffering from bacterial diarrhea in 1919, and it proved to be effective with no adverse effects (Kashani et al., 2018). This was the start of phage treatment. There are certain type of Lysins, also known as Endolysins, are enzymes that are encoded in bacteriophages. They work by dissolving the cell wall of the host organism, which makes it possible for bacteriophage progenies to be released (Rodrguez-Rubio et al., 2016)

(Gondilet al., 2020). During the course of his research on bacteriophages, Frederick W. Twort discovered that lysates of phage include catalytic activity that may stimulate bacterial lysis in vitro (Twort et al., 1915). Twort reported what was possibly the first evidence of endolysins in his landmark work, indicating that there seemed to be a heat-labile, non-transmissible property that created translucent regions of lysis (Twort, 1915). Endolysins are produced towards the completion of the phage lytic cycle, in contrast to virion-associated peptidoglycan hydrolases (VAPGHs) (Rodrguez-Rubioet al., 2016). Peptidoglycan hydrolase is a comprehensive term for a collection of lytic enzymes that act on peptidoglycan from bacteria and are classified according to their origin. An "autolysin" is a PG hydrolase that the bacterial cell produces and regulates for PG growth, division, maintenance, and repair. An "exolysin," on the other hand, is an enzyme produced by a bacterial cell that works to lyse the PG of a different strain or species inhabiting the same biological niche (Schindler & Schuhardt, 1964). Artilysins are antibacterial proteins that have been created. Their mechanism is mostly based on endolysins, although it has been refined and enlarged via bioengineering (Antibiotic alternatives, 2022).

Phage lytic enzymes, also known as lysins, are extraordinarily efficient molecules that have developed for this purpose over millions of years. These enzymes are intended to attack one of the four primary bonds in the peptidoglycan and target the integrity of the cell wall. Ernest Hanbury Hankin, a British bacteriologist, produced the first documented finding of antibacterial-like action connected to bacteriophages in 1896. (Trudil & David, 2015). F. D. Reynals, a phage researcher, had confirmed Twort's lytic findings by 1926. He did the same experiments on Gram-positive and Gram-negative organisms and saw that they were completely unique to Gram-positive species. This proved that Twort's discoveries were correct (Reynals, 1926). Alice Evans established the analytical field of phage typing in 1934 after using phage to classify various bacterial species (Evans, 1934). Later on, W. R. Maxted studied the rationale for nascent lysis that was observed by Evans and extracted a lytic component from bacteriophage filtrates in order to establish its origin and role in the phage lytic system (Maxted, 1957). Richard Krause gave the Evans B563 phage the designation C1 in 1957. This was done because of the phage's shown preference for Group C streptococci. The first scientist to partially purify C1 lysin was Krause, who did so in his research (Gondil et al., 2020). As a consequence of his thesis work at the McCarthy lab in 1971, Vincent Fischetti produced a very pure C1 lysin, enabling more accurate research on how the surface proteins of Gram-positive organisms' link to the cell wall (Gondil et al., 2020). The term "Endolysin" was then defined as any one of a number of unrelated types of enzymes, such as muramidase, amidase, or transglycosylase, that attack either the peptide bonds (such as amidases) or glycosidic bonds (such as muramidase and transglycosylases) that give the peptidoglycan its structural stability (Young, 1992). Later on, in the early 2000s, endolysins were put to use in the medical treatment of in vivo bacterial infections. Nelson was the first person to publish a study on the effectiveness of endolysin C1 as a preventative agent against upper respiratory group A streptococci in an in vivo model (Nelson et al., 2001).

An oral dose of an endolysin from the streptococcal C1 bacteriophage offered protection against upper respiratory colonization in mice that had been exposed to *Streptococcus pyogenes* (group A streptococci). Based on these findings, scientists developed the word "enzybiotic" to define the therapeutic potential of any bacteriophage-derived endolysins, not just streptococcal endolysins

(Nelson & Daniel, 2012). Endolysin clinical studies were authorized in 2013, including a phase 1 evaluation of the safety, pharmacokinetics, and pharmacodynamics of endolysin SAL-1-based medicines intended to treat antibiotic-resistant staphylococcal infections (Jun et al., 2013). Later on, a phase I/II trial employing the engineered chimeric endolysin Staphefekt was conducted (Abdelkader et al., 2019).

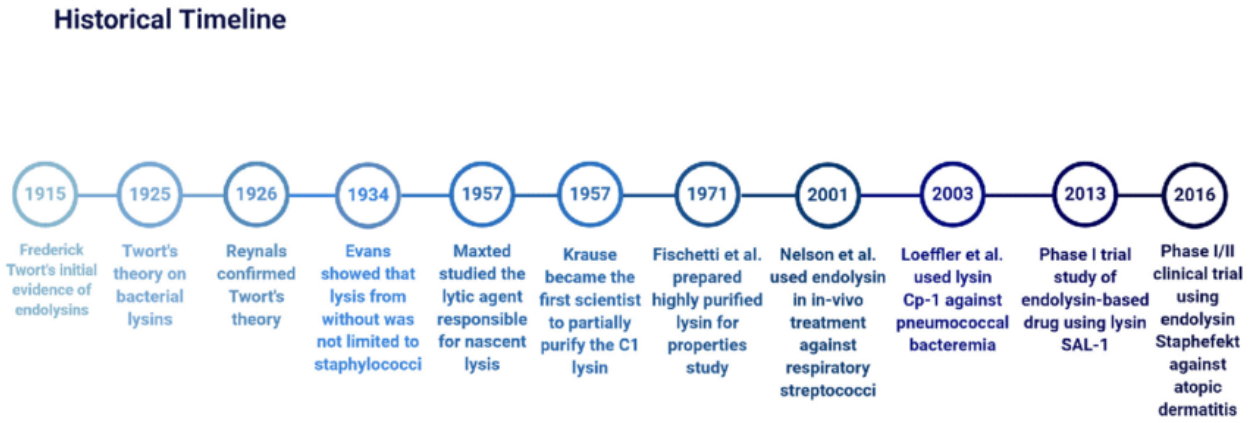

Figure 3. A timetable illustrating a brief summary of the history of endolysin research (Abdelrahman et al., 2021).

3.2 Why Lysin

Lysins are preferred over antibiotics. Lysin may be used to treat super infections because of its benefits. Lysins are cell-wall-hydrolyzing enzymes that destroy bacteria on contact (Fischetti, 2008). Generally, lysins destroy only the bacteria from which they were made. Streptococcal phage enzymes kill streptococci, whereas pneumococcal phage enzymes kill pneumococci (Loeffler et al., 2001). (Nelson et al., 2001). A lysin from a group C streptococcal phage (PlyC) kills group C streptococci, as well as groups A and E streptococci, the bovine pathogen *Streptococcus uberis*, and the horse pathogen *Streptococcus equi*, but has no impact on human oral streptococci and other gram-positive bacteria.

Lysin is specific to the particular pathogen (Briers et al., 2009; López et al., 1997; Schuch et al., 2002; Loessner et al., 2002), which preserves the normal microbiota (Cheng et al., 2017). Lysins are host-specific. Due to their broad-spectrum mode of action, most antibiotics were researched as treatments. One antibiotic may be authorized and administered for several diseases. One major problem is the collateral harm to the microbiota. Antibiotics often kill beneficial commensals along with pathogens. Off-target effects may cause dysbiosis in the microbiome. This causes metabolic problems, diabetes, malnutrition, and Clostridium difficile infection (Langdon et al., 2016). Endolysins target a single pathogen in a group of bacteria, sparing commensals.

These enzymes target a crucial and well-conserved structural component, peptidoglycan, which cannot be readily manipulated without affecting viability (Briers et al., 2014; Gilmer et al., 2013; Kusuma et al., 2007). Lysins are active regardless of the bacterial physiological condition (Blázquez et al., 2016; Vázquez et al., 2017). If the bacterial physiological condition changes, that particular lysin will still be functional.

Multidrug-resistant bacteria withstand many antibiotics (MDROs for short) (Gutiérrez et al., 2018; Briers et al., 2014; Loeffler et al., 2001; Dez-Martnez, 2015). Multidrug resistance enhances antibiotic resistance. When many antibiotics select for the same resistant bacteria or plasmids, lowering one antibiotic's usage will not diminish resistance. Two primary ways may cause multidrug resistance in bacteria. First, bacteria may acquire numerous drug-resistance genes in a single cell. Typically, resistance plasmids accumulate. Second, increased expression of multidrug efflux pumps-coding genes may cause multidrug resistance (Nikaido, 2009).

Most antibiotics disrupt a critical bacterial metabolic process, causing cell degradation and death (Hans et al., 2016). Phage-derived lysins target one of four primary peptidoglycan linkages, degrading bacterial cell wall peptidoglycan. The cell wall maintains bacterial form and resists osmotic pressure. Lysin accelerates bacterial osmotic lysis by generating holes in the cell wall within seconds of contact, killing the target organism quicker than other antibiotics. Bacteria may use a variety of metabolic pathways. A blocked path may be replaced with a different route. Antibiotic resistance is a fast-moving phenomenon. Lysins generated from phages are not influenced. No lysin-resistant strains have been reported despite repeated lysin exposure (Rios et al., 2016). These lysins are effective in killing bacteria, despite the organism's resistance to antibiotics. Lysins are effective against multidrug-resistant bacteria such as Vancomycin-resistant *Enterococcus faecium* (VREF). Lysins have a unique bactericidal mechanism, unlike antibiotics. Bacteria resistant to lysin are very rare, since these enzymes have been developed over millions of years by phage in conjunction with their bacterial hosts. Lysins may have evolved to target important bacterial peptidoglycan molecules. They may synergize with other lysins or antibiotics to prevent resistance and boost therapeutic efficacy. Some lysins function in synergy in vitro and in vivo with others or particular antibiotics. (Jado et al., 2003; Becker et al., 2008; Loeffler & Fischetti, 2003). In a mouse sepsis model, Cpl-1 and Pal decreased bacteremia more than either lysin alone (Jado et al., 2003; Loeffler & Fischetti, 2003). In another in vitro experiment, LysK and lysostaphin showed antibacterial synergy (Becker et al., 2008). Lysins and antibiotics showed promising results in, in vitro synergy. Two pneumococcal enzymes, Cpl-1 and LytA, are utilized with penicillin, gentamicin, cefotaxime, and moxifloxacin. Antibiotics enhanced enzyme activity in the vast majority of these cases. When combined with vancomycin or teicoplanin, staphylococcal lysin MV-L demonstrated increased action against VISA strain Mu50 (Rashel et al., 2007; Rodrguez et al., 2007; Duarte et al., 2021).

The production of bacterial biofilms is a survival strategy that promotes bacterial pathogenicity and antibiotic resistance. The tolerance of biofilms to antimicrobials and human immunity has prompted the research for alternative antibiofilm and antimicrobial-resistant strains techniques. Lysins destroy mucosal and biofilm-growing bacteria. Phage lytic proteins have remarkable antibiofilm characteristics. In recent research, the virulent bacteriophage phiIPLA-RODI and the

phage-derived lytic protein CHAPSH3b were combined to remove *Staphylococcus aureus* bacterial growth. The combined application of the two antimicrobials resulted in larger significant reduction in viable cell counts than each therapy used alone. *Staphylococcus aureus* and mixed-species biofilms were completely eliminated by lysin CF-301 from plastic, glass, surgical mesh, and surgical instruments. When coupled with the cell wall hydrolase lysostaphin, antibiofilm performance improved (Schuch et al., 2017). Similarly, phage endolysin LysCSA13 exhibited significant efficiency in eliminating staphylococcal biofilms on polystyrene, glass, and stainless steel, reducing biofilm mass by 80–90%. (Cha et al., 2019).

Chapter 4 Current Development of Lysin in Respiratory Tract infections

4.1 Lysin to treat Gram-positive infections

Hollins, a second category of phage-encoded proteins, are required for lysins to access their peptidoglycan substrate in the cell wall. These proteins create holes in the cytoplasmic membrane, allowing lysin molecules to move into the periplasm and make contact with the peptidoglycan substrate. Including both Gram-positive (such as Streptococcus, Lactococcus, Listeria, and Bacillus) as well as Gram-negative (such as Salmonella, *E. coli*, and Haemophilus) bacteria, the complex procedure seems to be the preferred phage lysis approach. Exogenous activity will be more efficient in Gram-positive bacteria than in Gram-negative bacteria based on differences in the cell wall structure of the two types of bacteria. In Gram-negative bacteria, the outer membrane will inhibit the phage lysins from reaching their peptidoglycan target. Previous studies have shown that Gram-positive phage lysins have a strong destructive effect on the cell walls of the bacteria that serve as their hosts. The cells were lysed from the outside by the environment when certain isolated lysins were exposed to the peptidoglycan of the target bacteria (Jofre & Muniesa, 2014).

4.1.1 *Streptococcus pneumoniae*

The human upper respiratory system, gastrointestinal tract, skin, and oral cavity are common places for streptococcus species to be found (Abranches et al., 2018). Infections may range from mild sore throats to life-threatening systemic conditions such as scarlet fever, streptococcal pharyngitis, necrotizing fasciitis, and toxic shock syndrome. Moderate sore throat infections are more common. One of these highly dangerous species, *Streptococcus pneumoniae*, has been linked to the development of pneumonia, otitis, meningitis, and even sepsis (Walker et al., 2013). An important human pathogen known as *Streptococcus pneumoniae* is exhibiting an increasingly high level of resistance to many kinds of antimicrobial drugs (Jacobs, 2004). (Schrag et al., 2004). There is evidence that several lysins, when combined, are capable of effectively eliminating many human pathogenic strains of *Streptococcus pneumoniae* (Vázquez et al., 2019).

Lysin Cpl-1 is a muramidase that has the ability to kill *Streptococcus pneumoniae* every quickly. It is a pneumococcal phage lytic enzyme (Loeffler et al., 2003; Djurkovic et al., 2005; Jado et al., 2003). Cpl-1 is being considered as a possible treatment agent for the treatment of multidrug-resistant pneumococci. The effectiveness of Cpl-1 against a strain of *Streptococcus pneumoniae*

that is resistant to multiple drugs was tested in the lab and in rats with endocarditis. The results of that experiment showed that giving higher doses of Cpl-1 could make it work better. In the series of experiments, rats were given what is known as a "high-continuous dosage" of Cpl-1, which is 25 times greater than the normal amount. With the high-dose Cpl-1 regimen, *Streptococcus pneumoniae* was killed quickly, and it seems like a lot of cell wall fragments were released, which made this cytokine secretion stronger. A control treatment with vancomycin was done to get a better idea of how well Cpl-1 killed bacteria and how much cytokine it made. Even though vancomycin worked, Cpl-1 seemed to work much faster. The use of Cpl-1 as a means of fighting *Streptococcus pneumoniae* infections not only in regions where the bacteria may easily move, such as the blood, but also in deep foci where the bacteria may be shielded by a protective physical barrier. But more research needs to be done to find out how much Cpl-1 humans need to take to reach therapeutic concentrations and if it is safe (Jado et al., 2003).

When tested similarly, it was shown that the lysin 23TH 48 produced by Streptococcal phage 23TH was effective against *Salmonella infantis* as well as a number of other isolates of *Streptococcus pneumoniae*. 23TH_48's cell wall binding domain, which is necessary for its killing activity and is identical to that of the autolysin LytA and the pneumococcal phage lysins Pal and Cpl-1. This protein's closest homolog is lysin from the *Streptococcus pneumoniae* phage Dp-1 (van der Kamp et al., 2020). This protein has been shown to be effective against several serotypes of *Streptococcus pneumoniae*, as well as *Streptococcus mitis* and *Streptococcus oralis* and it has a broad lytic spectrum. Within only one hour, 23TH_48 lysin brought about a significant decrease in the number of *Streptococcus pneumoniae* cells by up to 4log10. The lysins Pal and Cpl-1 both produced comparable results in the past when tested for their ability to reduce turbidity (Loeffler et al., 2003; Loeffler, 2001). Under the same conditions, the cell counts of a *Streptococcus pneumoniae* serotype 19F strain were reduced by 4.2 log10 CFU/mL when treated with the Cpl-1 lysin, whereas the R6 strain had a reduction of 3.2 log10 CFU/mL (Loeffler et al., 2003). Cpl-1 lysin at a concentration of 5 ng/L was incubated for 60 min at 37°C with the *Streptococcus pneumoniae* R6 strain to eradicate the culture (Dez-Martnez et al., 2014). As a potential pneumococcal infection therapy, phage lysin 23TH 48 might be a viable alternative for further study. There is a chance that combining 23TH 48 with other lysins, bacteriocins, or antibiotics might enhance its lytic properties. Combinations like this might boost killing efficiency while also broadening the bacterial range of action. Using this technique, several *Streptococcus pneumoniae*-targeting lysins have been effective (Van der Kamp et al., 2020).

Pneumococcal phage MS1 encodes an additional lysin, MS1ys, which is a unique natural endolysin. MS1ys is the name given to the gene that was found to be ms1_61, which was designated as a lysin. MS1ys is a modular endolysin of 295 amino acids. Research using bioinformatics showed that it has both N-acetylmuramoyl-alanine amide amidase activity as well as a CBD that included five cell-wall binding repetitions (Silva et al., 2020). It was shown that MS1ys had a particular antibacterial impact on *Streptococcus pneumoniae*, including strains isolated from otitis media in children. According to research (Kurola et al., 2010), the majority of clinical pneumococcal strains express a capsule.

Protein engineering has enhanced the characteristics of *Streptococcus pneumoniae* endolysins in recent years. It has, for example, boosted their antibacterial activity, plasma half-life, and capability of passing through the membrane of the negatively charged bacterial cells. The incorporation of additional residues (Cpl-1 dimer) (Silva et al., 2020; Resch et al., 2011), the swapping of the charge of the CBD (Cpl-7S) (Dez-Martnez et al., 2013), and fusion and shifting of domains (Cpl-711, PL3) (Blázquez et al., 2016) were responsible for these enhancements (Diez-Martinez et al., 2015). Natural MS1ys is not as effective as manufactured endolysins, but it has exceptional properties against *Streptococcus pneumoniae* (Silva et al., 2020).

4.1.2 *Mycobacterium spp.*

The gram-positive pathogen *Mycobacterium tuberculosis*, which is the etiological agent of TB and represents a severe danger because of the increase in variants of the bacteria that are resistant to antibiotics, Mycobacterial infections continue to be among the world's deadliest and most devastating illnesses, with tuberculosis (TB) being the most frequent kind. TB is a respiratory infectious disease that is brought on by direct contact with the acid-fast bacterium known as *Mycobacterium tuberculosis* (Mtb). The ever-increasing prevalence of antibiotic resistance has turned this illness into a serious threat to public health (Mulleret al., 2013). An estimated 10 million people were diagnosed with tuberculosis in 2018, with a half million of those cases being resistant to rifampicin (of which 78% had multi-drug resistant TB).

The alarming growth of multidrug-resistant and exceptionally drug-resistant tuberculosis has raised the requirement for creating innovative and efficient antimycobacterial treatments (Marrakchiet al., 2014). This is the case despite the fact that curative chemotherapy is now available (Mitnicket al., 2009).

When it comes to the development of lysin-based treatments, the acid-fast *Mycobacterium tuberculosis* strain is still relatively understudied. It's possible that this is because of a peculiarity in the structure of CW of Mycobacterium has a dense PG layer that is crosslinked to arabinogalactan and sterified by mycolic acids (Squeglia et al., 2018). Mycobacteriophages each have their own unique complement of lytic enzymes, which they use to breakdown the complex cell membrane of their mycobacterial hosts in order to free the viral offspring they carry. Depending on the structure of mycobacteriophages, their lytic domains include two separate lytic enzymes: a standard PG hydrolase, also known as LysA, and mycolyl-arabinogalactan esterase (LysB), which breaks down proteins the ester link linking mycolic acid to the arabinogalactan-PG layer. As a consequence of this, the layer of mycolic acid becomes detached from the cell wall, leaving the cell susceptible to osmotic shock and, ultimately, lysis (Payne et al., 2012).

Several mycobacteriophage-derived hydrolases and lysis enzymes that were developed by mycobacteriophages have been used in several in vitro studies. These experiments have shown largely positive outcomes that suggest either growth inhibition (Grover et al., 2014) or that pathogenic mycobacteria may be eliminated by using the lysis enzymes generated by mycobacteriophages, which target major cellular membrane components (Lai et al., 2015). Additional studies are currently in process.

4.1.3 *Staphylococcus aureus*

Infectious diseases such as infections of the skin, endocarditis, bronchitis, meningitis, and osteomyelitis may be caused in people and animals by the Gram-positive bacteria *Staphylococcus aureus*, which can cause staphylococcal food poisoning as well as other infectious diseases (Lowy, 1998; De Lencastre et al., 2007). *Staphylococcus aureus* is a common bacterium that causes different kind of infections in the respiratory system, from asymptomatic colonization to fulminant necrotizing pneumonia (Parker & Prince, 2012). *Staphylococcus aureus*, gram-positive cocci that move to the lungs via the blood from other infected areas, most often the skin, causes staphylococcal pneumonia.

The majority of *Staphylococcus aureus* lysins examined have two cell domains (NAM-amidase and endopeptidase) as well as a cell wall binding domain SH3b (Schmelcher et al., 2015; Sass et al., 2007; Pritchard et al., 2004). It has been hypothesized that certain cell wall binding domains recognize the pentaglycine peptide cross bridge, even if the actual connection between the cell wall binding domain and the structures to which these domains attach has not always been shown (Gründling et al., 2006) or CW-associated glycopolymers (Daniel et al., 2010). Antibiotics are the only treatment option for *Staphylococcus aureus* pneumonia, which is a very dangerous condition due to the organism's extraordinary potential to build resistance to antimicrobials. The pore-forming cytotoxin S. aureus alpha-hemolysin is required for the development of pneumonia (Ragle et al., 2010).

An analysis revealed the antibacterial efficiency of LysP108 in vitro and in vivo, in addition to its potential in combination with antibiotics. This paves the way for a new strategy to address the rising problem of bacterial drug resistance. LysP108, although being linked to a significant number of previously discovered endolysins, has a unique amino acid sequence and domain structure, according to sequence homology analyses.

LysP108's lytic activity was assessed, and it was discovered that LysP108 caused lysis of XN108, with the healthy cell decreasing by around two log units after the therapy had been applied for thirty minutes. The Structural Equation Modeling (SEM) research revealed further proof of LysP108's lytic ability. Figure 4 illustrates how MRSA (XN108 and SCCmec V) cells were gradually lysed in vitro after being exposed to LysP108 for varying amounts of time.

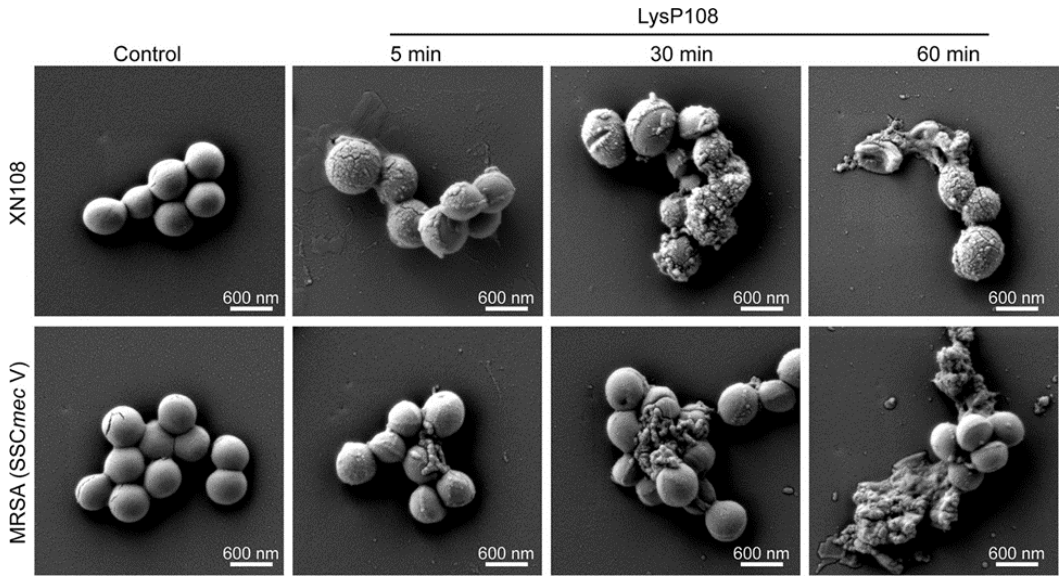

Figure 4: MRSA strains (XN108 and SCCmec V) after varied times of LysP108 and PBS incubation are shown in SEM micrographs (Lu et al., 2021).

A viable bacterial immunofluorescence tool was used to demonstrate endolysin's additional bactericidal action, and the results were then analyzed under a fluorescence microscope. Another figure showed that the red fluorescent color channel only showed the dead organisms, but the green, fluorescent color channel showed all the organisms. The co-occurrence of the two fluorescence signals was indicative of the fact that LysP108 was successful in eliminating the majority of the bacteria. It was estimated that around 90% of the bacteria would be killed by the treatment.

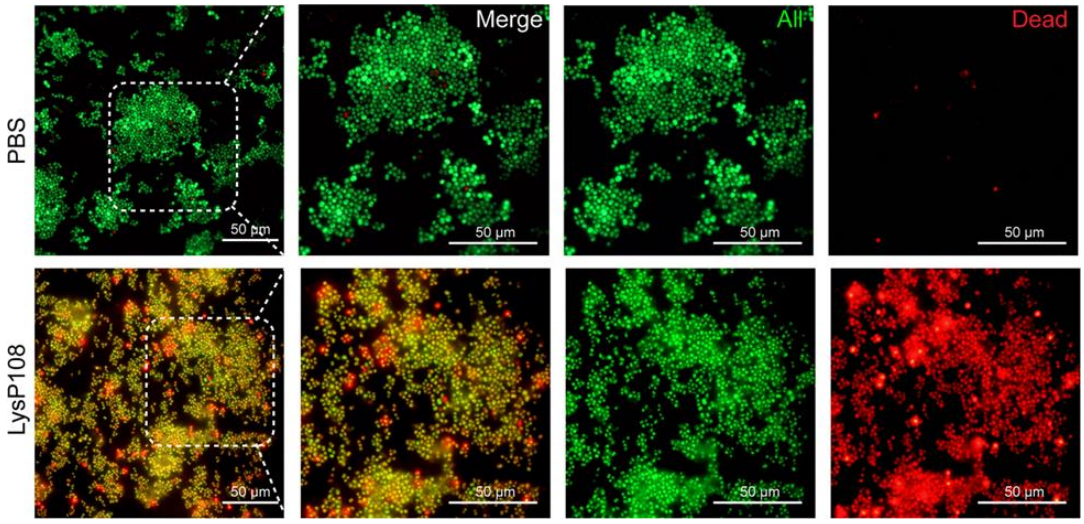

Figure 5: Fluorescent images showing the staining of viable bacteria. Red color indicates dead bacteria whereas green color indicates, all living bacteria (Lu et al., 2021).

The results of another study demonstrated that the novel chimeric endolysin known as Lys109 exhibited a higher level of bacterial cell lytic activity that is superior to that of its parental endolysins in combating staphylococcal biofilms and planktonic cells. Additionally, it showed noticeably enhanced results in the elimination of *Staphylococcus aureus* strains from milk and stainless-steel surfaces. These results suggest that a novel hybrid endolysin with enhanced activity and fluidity may be produced by random domain shifting, and that this hybrid endolysin has a substantial amount of potential for the future as an effective antibacterial.

4.1.4 *Streptococcus pyogenes*

Streptococcus pyogenes (group A streptococci) is the most prevalent cause of purulent respiratory tract and skin infections in humans, affecting about 720 million individuals annually. (Carapetis et al., 2005; Cunningham, 2000). *Streptococcus pyogenes* is the leading cause of infections in the upper respiratory tract. The leading cause of bacterial pharyngitis (Brouwer et al., 2016) is among the few infections in humans that remains universally responsive to penicillin (M H Macris, 1998). In addition of 2.5 million cases of streptococcal-mediated pharyngitis are reported yearly in the More than 80% of these incidents involved youngsters under the age of 15 in the United States. Furthermore, Up to 35% of pharyngitis individuals who get penicillin do not have success in eliminating streptococci (Pichichero, 1998), and in direct contact situations, such childcare facilities, rates as high as 50% have been seen (Feldman et al., 1987).

Streptococcus pyogenes bacteria may be resistant to macrolides even after their remaining susceptibility to penicillin, with resistance rates varied widely across regions and being especially prevalent in Italy (Cornaglia & Bryskier, 2004; Varaldo et al., 1999; Creti et al., 2005). Cigarette smoke exposure, low birth weight, and poor socioeconomic status are all known risk factors for *Streptococcus pyogenes* related upper respiratory tract infections (Steer et al., 2007; Danchin et al., 2007; Nandi et al., 2001). *Streptococcus pyogenes* infections often do not improve with antibiotic therapy, linked to severe throat carriage and persistent infections. Such failures cannot always be explained by the presence of antibiotic resistance determinants, and it has been hypothesized that *Streptococcus pyogenes* enters epithelial cells to avoid antibiotic treatment. Consequently, lysin is a viable alternative to antibiotics for treating these resistant bacteria. The lysin known as PlyC, which is very uncommon and is a multimeric enzyme, is the most relevant example of a lysin that targets this particularly group A streptococci (GAS) (Nelson et al., 2001; Nelson et al., 2006).

Furthermore, it has been demonstrated that PlyC is capable of penetrating respiratory tract epithelial cells and destroying *Streptococcus pyogenes* cells that are located inside these cells (Shen et al., 2016). This intrinsic activity overcomes one of the most fundamental drawbacks of antibiotic therapy for streptococcal throat infections. This barrier relates to the fact that bacteria may defend themselves from antibiotics by invading cells. Other bacterial lysins that have been shown to be effective against *Streptococcus pyogenes* include PlyPy (Lood et al., 2014) and Cpl-7S (Dez-Martnez et al., 2013), a pneumococcal phage-derived lysin with a wide range. Furthermore, it is known that group B streptococci may cause serious pneumonia in infants (Heath

et al., 2017). At least one attempt has been conducted, utilizing PlyGBS lysin, to eliminate group B streptococci from the oropharynx of mice (Cheng et al., 2005).

Gram-positive bacteria susceptible to Lysins

			Methodology used		
Species	**Lysin/Phage**	**Susceptible bacteria tested**	**In Vitro**	**In Vivo**	**References**
S. Pneumoniae	Pal/Dp-1	Pneumococci and relatives	Biofilm; synergy with Cpl-1	Colonization and sepsis (mice)	(Loeffler et al., 2001), (Loeffler & Fischetti, 2003), (Domenech et al., 2011)
	Cpl-1/Cp-1	Pneumococci and relatives	Biofilm; synergy with Pal and antibiotics; cell culture	Colonization, otitis, pneumonia, sepsis (mice)	(Loeffler & Fischetti, 2003) (Witzenrath et al., 2009)
	LytA	Pneumococci and relatives	Biofilm	Sepsis (mice)	(Domenech et al., 2011) (Díez-Martínez et al., 2013)
	Cpl-7/Cp-7	Streptococci; other G+	Biofilm	/	(Domenech et al., 2011) (Díez-Martínez et al., 2013)
	Cpl-7S	Streptococci; other G+	Cell culture	Colonization (mice), pneumococcal infection (zebrafish)	(Corsini et al., 2018) (Díez-Martínez et al., 2013)
	Cpl-711	Pneumococci and relatives	Biofilm; synergy with antibiotics; cell culture	Colonization and sepsis (mice), pneumococcal infection (zebrafish)	(Díez-Martínez et al., 2015) (Corsini et al., 2018) (Letrado et al., 2018)
	PL3	Pneumococci and relatives	Biofilm	Pneumococcal infection (zebrafish)	(Blázquez et al., 2016)

			Methodology used		
Species	**Lysin/Phage**	**Susceptible bacteria tested**	**In Vitro**	**In Vivo**	**References**
S. aureus	Lysostaphin	Staphylococci	Biofilm; synergy with LysK; CHAPK and antibiotics; controlled release	Sepsis and colonization (mice, rats)	(Kusuma et al., 2007), (Schmelcher et al., 2015), (Kokai-Kun et al., 2003)
	LysK/K	Staphylococci	Biofilm; complex with polycationic peptides	/	(Schmelcher et al., 2015) (O'Flaherty et al., 2005) (Filatova et al., 2013)
	CHAPK	Staphylococci	Biofilm; synergy with lysostaphin; controlled release	Colonization (mice)	(Polak et al., 1993) (Fenton et al., 2010) (Fenton et al., 2013)
	ClyS	Staphylococci	Synergy with oxacillin and vancomycin	Colonization and septicemia (mice	(Daniel et al., 2010)
	SAL-1/SAP-1	Staphylococci	Biofilm	Bacteremia (mouse), toxicity and pharmacokinetics (rats, dogs, monkeys), pharmacokinetics and pharmacodynamics (healthy humans)	(Jun et al., 2014) (Jun et al., 2013)
	P128	Staphylococci	Biofilm; cell culture; synergy with antibiotics	Colonization and sepsis (rats)	(Paul et al., 2011) (Channabasappa et al., 2018)
	LysGH15/GH15	Staphylococci	Biofilm	Sepsis and pneumonia (mice)	(Xia et al., 2016), (Zhang et al., 2018)
	CF-301 (PlySs2)/S. suis 9/1591 prophage	Staphylococci	Biofilm; synergy with antibiotics	Sepsis (mice)	(Gilmer et al., 2013) (Schuch et al., 2013) (Schuch et al., 2017)
	ClyF	S. aureus, S. pyogenes, S. pneumoniae; other G+	Biofilm	Sepsis (mice)	(Yang et al., 2017)

			Methodology used		
Species	**Lysin/Phage**	**Susceptible bacteria test**	**In Vitro**	**In Vivo**	**References**
S. pyogenes (GAS)	PlyC/C1	GAS and other streptococc	Biofilm; cell culture (intracellular killing of GAS)	Colonization (mice)	(Nelson et al., 2001), (Nelson et al., 2006), (Shen et al., 2013)
	PlyPy/MGAS31	GAS and other streptococci	/	Sepsis (mice)	(Loeffler & Fischetti, 2003) (Witzenrath et al., 2009)
S. agalactiae (GBS)	PlyGBS	GAS, GBS and other streptococci	/	Colonization (mice)	(Cheng et al., 2005), (Cheng & Fischetti, 2007)

			Methodology used		
Species	**Lysin/Phage**	**Susceptible bacteria test**	**In Vitro**	**In Vivo**	**References**
Mycobacterium sp.	LysB/Ms6	Mycobacteria	Growth inhibition with surfactants	/	(Grover et al., 2014) (Gil et al., 2010)
	LysB/Bxz2	Mycobacteria	Growth inhibition with surfactants	/	(Grover et al., 2014)
	LysA/BTCU-1	Mycobacteria	Cell culture	/	(Lai et al., 2015)
	LysB/BTCU-1	Mycobacteria	Cell culture	/	(Lai et al., 2015)

Table 2: Active lysins against Gram-Positive pathogens.

4.2 Lysin to treat Gram-negative infections

Phage and their lysins have medicinal promise since they can rapidly lyse target bacteria with little impact on normal flora (Sillankorva et al., 2011). Endolysins target gram-positive bacteria's naturally exposed peptidoglycan layer to lyse their cells. Gram-negative bacteria with an outer membrane are less susceptible to endolysin treatment. Gram-negative endolysin therapy is challenging (Schmelcher et al., 2012). Gram-negative bacteriophage endolysins are unable to effectively destroy Gram-negative strains because they are coated in a peptidoglycan that is impenetrable to them (Yan et al., 2019). Lipopolysaccharide molecules are held together by phosphate-linked acidic sugars. Endolysins can't pass through Gram-negative bacteria's inner peptidoglycan layer (Nikaido, 2003). Pre-treatments are utilized to bypass cell membrane barriers. EDTA may permeate gram-negative cell membranes. EDTA removes divalent cations from the outer membrane, allowing lysin to invade (Schmelcher et al., 2012). The addition of membrane destabilizing chemicals such as poly-l-lysine or polymyxin B, as well as modifying the lysins to incorporate highly charged or hydrophobic residues, are two additional methods that may be used to improve the permeability of membranes to lysins (Guo et al., 2017; Briers et al., 2014; Briers et al., 2011) (Paradis-Bleau et al., 2007) (Shavrina et al., 2016). High hydrostatic pressure (HHP) kills bacteria and enzymes. HHP has been used to develop Gram-negative bacteria sensitive to bacteriocins and antimicrobial peptides by adjusting time and pressure (Briers & Lavigne, 2015). Artilysin, a molecularly engineered endolysin, was employed against gram-negative bacteria (Briers et al., 2014). After adding a combination peptide, modified endolysin might be able to kill multidrug-resistant cells.

4.2.1 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is one of the Gram-negative bacteria that has great metabolic flexibility, allowing it to flourish in a wide variety of environments (Silby et al., 2011). *Pseudomonas aeruginosa* highly effective opportunistic pathogen that causes a broad spectrum of acute and chronic illnesses (Winstanley et al., 2016) and is a prominent source of nosocomial infections across the globe (Spencer, 1996). (Koulenti et al., 2009). Bacteria may be killed by lysins because they dissolve the peptidoglycan membrane that is located in the cell wall, which ultimately leads to osmotic lysis. Because the peptidoglycan target is exposed on the surface of gram-positive bacteria, it is able to be attacked by lysins that are introduced from the outside. On the other hand, the outer membrane of gram-negative bacteria prevents the majority of lysins that have been delivered from the outside from reaching their peptidoglycan target. Because of the restricted permeability of its outer membrane, *Pseudomonas aeruginosa* is naturally resistant to numerous antibiotic classes (Nicas & Hancock, 1983) (Hancock, 1998). It can also develop resistance to all relevant antibiotics via mutations or the acquisition of additional genetic material, significantly reducing the therapy options available (El Solh & Alhajhusain, 2009). Some recent initiatives have concentrated on the designing of the enzymes themselves, giving rise to the "artilysin" concept, because of the potential issues with therapies that include the simultaneous administration of lysins and permeabilizing medicines (Briers et al., 2014). In the initial days of testing against *Pseudomonas aeruginosa*, lysins like EL188 were the only ones that could kill bacteria when they

were combined with membrane permeabilizers like EDTA, polycationic agents (Briers et al., 2007; Briers et al., 2011). Lysins were fused to cationic antimicrobial peptides (AMPs), and these fusions had permeabilizing activity, enabling them to cross the outer membrane (OM) of *Pseudomonas aeruginosa* and degrade the PG layer in vitro and in vivo (Briers et al., 2014). Lysin KZ144 and the sheep myeloid AMP-29 (SMAP-29) were combined to make Art-175, an artilysin. The thermostability of the resulting hybrid was improved by point alterations of several cysteine residues (Walmagh et al., 2014). Additionally, Art-175 inhibited the formation of resistant strains, bacterial subpopulations that often develop after anti-infective therapy and are partially resistant to antibiotics (Fisher et al., 2017). There are presently no lysins that can lyse Gram–negative bacteria on their own, despite bioengineering efforts. These lysins have AMP-like parts, which are peptides with a net positive charge and an amphipathic secondary structure. These parts make the OM less stable (Düring et al., 1999). One of the earliest examples of a lysin containing a natural cationic peptide being employed as an enzybiotic was the Bacillus amyloliquefaciens phage lysin Lys1521, which was capable of lysing *Pseudomonas aeruginosa* cells (Morita et al., 2001). OBPgp279 (Walmagh et al., 2013) and LysPA26 are two other lysins produced by *Pseudomonas aeruginosa* that have their own distinct anti-Gram-negative action (Guo et al., 2017).

4.2.2 *Klebsiella spp.*

Klebsiella pneumoniae is an Enterobacteriaceae Gram-negative pathogen (Rocket et al., 2014). *Klebsiella pneumoniae* may cause infection in people and animals with compromised immune systems or unbalanced microbiota, producing severe morbidity and death rate (Brisse & Duijkeren, 2005; Yang et al., 2021). Strains of this species may colonize the nasopharynx and GI tract (Martinet al., 2018) and cause a wide range of infections, including pneumonias, UTIs, bloodstream infections, and liver abscesses (Paczosa & Mecsas, 2016; Fang et al., 2007; Siu et al., 2012). *Klebsiella pneumoniae* is an issue in organ transplants and medical equipment. The gram-negative bacteria Klebsiella are an opportunistic pathogen that may cause nosocomial infection (Podschun & Ullmann, 1998; Fursov et al., 2020). When treating bacterial pneumonia, antibiotics are often the first line of defense. Incorrect use of antibiotics, on the other hand, has contributed to widespread *Klebsiella pneumonia* resistance (Pires et al., 2020). The large auxiliary genome of *Klebsiella pneumonia,* which appears as plasmid and chromosomal gene loci and contains antibiotic-resistant genes, is the cause of the organism's broad antibiotic resistance (Bi et al., 2015; Fang et al., 2020). The features of the biofilm that *Klebsiella pneumonia* forms were shown to considerably alter both its virulence and its resistance to antimicrobials (Vuotto et al., 2017).

LysECD7 demonstrated extensive antibacterial action against planktonic forms and was suggested for further study (Antonova et al., 2020). In 2014, the MDR *Klebsiella pneumoniae* strain Ts 141-14 was first identified from an individual patient's urination who was being treated at the Moscow Medical and Rehabilitation Center. *Klebsiella pneumoniae* strain Ts 141-14 was selected as a model because of its biofilm generation and virulent behavior in mouse models. Stepanovic et al. (2007) tested the in vitro activity of LysECD7 against developing and mature biofilms on the *Klebsiella pneumoniae* Ts 141-14 strain, a robust biofilm producer. They also tested endolysin's activity with amikacin, the only antibiotic active against the Klebsiella test strain. Crystal violet

staining was used to analyze LysECD7's antibacterial activity against *Klebsiella pneumoniae* strain Ts 141-14 biofilm. LysECD7 at 1000 and 3000 g/mL (62 and 186 M) reduced biofilm development by 74% and 79%, respectively (Fursov et al., 2020).

Antimicrobial activity was reported in vitro for LysCA and LysG24. Endolysin lysed lung pathogenic *Klebsiella pneumoniae* somewhat (LPKP). EDTA, which chelates gram-negative bacteria's outer membrane, increased endolysin's antimicrobial action. LysCA-EDTA combo has the greatest lysis capacity (Pires et al., 2020). It's possible that the cecropin A residues and EDTA work together to damage gram-negative bacteria's outer membranes, facilitating endolysin lysis of the host bacterium. This synergistic impact has been validated in certain research, although the particular molecular mechanism is still being investigated (Yuksel et al., 2018; Lai et al., 2020; Huang et al., 2021). Clinical and pathological observations of endolysin's effect on a mouse pneumonia model. By introducing a bacterial dosage through the intranasal method, LPKP was employed to create an experimental pneumonia model. Clinical symptoms and lung pathological section data showed that treatment group mice had much less damage than the control group animals (Pires et al., 2020). In addition, LysCA and LysG24 may effectively limit the development of the host bacteria LPKP in vivo and reduce inflammation in the lung tissue (Peng et al., 2019; Dhungana et al., 2021). In contrast, LysCA exhibited greater therapeutic efficacy based on clinical symptoms and bacterial burden in mouse lungs. By putting together various endolysin-containing peptides, a powerful antibacterial lysin may be created (Oliveira et al., 2018; Murray et al., 2021). LysG24 and LysCA endolysin exhibited no clinical harm in mice. LysG24 and LysCA have good in vivo and in vitro antibacterial activity and might treat LPKP-induced infections. In BALB/C mice, LysG24 and LysCA were safe. LysCA with cecropin A residues had superior antibacterial and environmental adaptability than LysG24. Potential candidates for use as antibacterial medications include endolysins that have been engineered (Lu et al., 2022).

The LysSAP26 lysin was investigated for antibacterial action with MDR bacteria, including Enterococcus faecium, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Escherichia coli* (Kim et al., 2020). LysAm24, LysAp22, LysECD7, and LysSi3 may swiftly eliminate Gram-negative bacteria in vitro, have a broad range of activity, and can dissolve biofilms and minimize wound and burn skin contamination, limiting local infections (Vasina et al., 2021).

4.2.3 *Escherichia coli*

Gram-negative *Escherichia coli* bacteria are common in the microbiota of the gut, where they play an important role in digestion (Conway et al., 2004). Essential oils produced from plants include antimicrobial components such as carvacrol, thymol, and eugenol (Perricone et al., 2015). Most antibacterial methods include outer membrane disruption (Marinelli et al., 2018). Because of their capacity to increase membrane permeability, carvacrol, eugenol, and thymol are responsible for the death of cells brought on by the disruption of the cytoplasmic membrane (Trombetta et al., 2005). (Yap PSX, 2021). In order to hasten the process of phage endolysin-mediated cell wall disintegration, *Escherichia coli* O157:H7 cells were cotreated with thymol or eugenol from essential oils before being exposed to endolysin LysECP26 (Park et al., 2021). Thymol perturbs

the plasma membrane's lipid percentage and alters membrane permeability, which may make cells larger by transferring water. By interfering with citrate metabolism, the antibacterial compound thymol suppresses the synthesis of ATP (Di Pasqua et al., 2006). Eugenol may harm cell membranes by altering fatty acid composition. LysECP26 has better access to the peptidoglycan layer with the compound-induced outer membrane breakdown to degrade it. These data imply that supplementing *Escherichia coli* O157: H7 NCCP 13930 cells with thymol or eugenol increases LysECP26's activity and generates morphological alterations. Combining LysECP26 with aromatic chemicals like thymol and eugenol improved bactericidal effectiveness. These phage lysis adjuncts are essential to rupture cell wall with endolysin and create *Escherichia coli* O157:H7 biocontrol agents (Park et al., 2021).

Endolysins fused to components that enable them to penetrate the peptidoglycan layer are another technique of targeting Gram-negative bacteria. The endolysin binding domain D8 of Bacillus amyloliquefaciens is fused with the phage lysin lysep3 of *Escherichia coli* lysin lysep3. Lysep3 alone can't break *Escherichia coli*'s outer membrane (Wang et al., 2017). The C-terminus of Lysep3 was modified to include a variety of hydrophobic amino acids, and these modifications were tested to see how they affected the ability of endolysins to penetrate the outer membrane of Gram-negative bacteria. A variety of endolysins were developed using a prokaryotic expression method that included hydrophobic amino acids at the C-terminal position (Yan et al., 2019). Five hydrophobic-modified endolysins were created by adding 3-12 hydrophobic amino acids to the *Escherichia coli* phage endolysin Lysep3. For example, different pH and endolysin concentrations affected lysis differently (Yan et al., 2019).

At the appropriate pH and concentration, endolysins that have been modified with hydrophobic amino acids kill *Escherichia coli* from the outside of the cell. Endolysin's ability to lyse proteins is improved by the C-terminal addition of hydrophobic amino acids. By making endolysin more hydrophobic at the C-terminus, it is possible to lyse Gram-negative antibiotic-resistant bacteria by attacking them externally (Yan et al., 2019). Because of an increase in the C-terminal hydrophobicity, endolysin was able to affect *E. coli* from outside the cell wall at pH 5.0 at a concentration of 1.75 g/L. EDTA could improve lysis. *Escherichia coli* was externally lysed by hybrid endolysins. It may provide a new way to combat gram-negative antibiotic-resistant infections (Yan et al., 2019). Gene-engineered phage endolysin can lyse gram-negative bacteria. *Escherichia coli* may be lysed by the hybrid endolysin Lysep3-D8 from outside the cell (Wang et al., 2017). Colicin A was combined with Lysep3 such that it could carry endolysin into the cell wall of bacteria and lyse them (Zhu et al., 2017). When combined with the D8 CWBD, it lyses bacteria from the outside. The D8 domain may rupture the bacterial outer membrane, enabling the enzyme to penetrate the peptidoglycan membrane. (2017). Endolysins, which are proteins that are hostile to water, are what cause *Escherichia coli* to die on the surface of the cell (Yan et al., 2019).

4.2.4 *Acinetobacter baumannii*

According to the CDC, *Acinetobacter baumannii* causes the most hospital-acquired illnesses worldwide (Lin et al., 2014). This bacterium has been labeled as a limited pathogen for a long time, but research has proven that it is a dangerous pathogen that may infect the epidermis,

bloodstream, urinary system, and other soft tissues (Peleg et al., 2008). The Gram-negative bacterium *Acinetobacter baumannii*, which presents a serious threat to human health, is responsible for several diseases. Due of their innate resistance and the overuse of antibiotics, certain *Acinetobacter baumannii* isolates are now tolerant to practically all known antimicrobials (Manchanda et al., 2010).

In general, it seems that lysins against G– bacteria are less selective than those against G+ bacteria. Because of this wider range, many lysins are able to kill a wide variety of pathogenic species. One example of this is the lysin LysPA26, which, in addition to *E. coli*, has the potential to lyse Gram-negative pathogens including *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* (Guo et al., 2017), or Art-175, which is similarly effective in eliminating *Acinetobacter baumannii* (Defraine et al., 2016). Due to its alarming growth in antibiotic resistance, this bacterium is a potential respiratory pathogen, particularly for immunocompromised and physically weak people. It has received much interest recently (Elhosseiny et al., 2018). Consequently, a number of enzybiotics, such as LysAB3 and LysAB4 (Lai et al., 2013), PlyAB1 (Huang et al., 2014), and LysABP-01, have been developed with a focus on their *Acinetobacter baumannii* killing potential (Thummeepak et al., 2016). In both planktonic and biofilm cultures, it was shown that PlyF307 was effective in killing *Acinetobacter baumannii* isolates, including MDR strains (Lood et al., 2015) and is the first case of an intact lysin that has been studied in a murine model with intrinsic anti-Gram-negative activity. Unsurprisingly, it was later discovered that the cationic peptide located in the lysin's C-terminal region was responsible for the extrinsic activity (Thandar et al., 2016). Similar sub-domain structural motifs have also been identified in other lysins; these motifs have the potential to permeate membranes but lack enzymatic activity. For instance, lysin LysAB2 (Lai et al., 2011) is a broad-spectrum enzybiotic with permeabilizing qualities that make it active against both Gram positive and Gram-negative bacteria (Lai et al., 2011).

Antimicrobial peptides generated from the C-terminal domain of LysAB2 showed strong antibacterial activity when it was tested on mice infected with *Acinetobacter baumannii* (Peng et al., 2017). Again, the natural sources endolysin may be a promising alternative antimicrobial agent against a number of MDR Gram-positive pathogens, according to a new bacteriophage endolysin, LysAB54, encoded by a new *Acinetobacter baumannii* bacteriophage p54. This new endolysin showed a broad spectrum of antibacterial activity against Gram-negative bacteria regardless of their growth phase (Khan et al., 2021). Due to its OMP-independent activity, LysAB54 is a potential option for the treatment of infections brought on by several drug-resistant Gram-negative pathogens. Wide-ranging lysins like LysSAP26 (Kim et al., 2020) and LysSS (Kim et al., 2020) have a lysozyme or glycoside hydrolase catalytic activity that breaks the β-1,4-glycosidic link between NAM and NAG (Ghose & Euler, 2020).

Gram-negative bacteria susceptible to Lysins

Species	Lysin/Phage	Susceptible bacteria tested	Methodology used	References
P. aeruginosa	Lys1521/B. amyloliquefaciens Phage	G–	Activity on intact bacteria	(Morita et al., 2001) (Muyombwe et al., 1999)
	EL188/EL	G–	Activity on permeabilized bacteria	(Briers et al., 2007) (Briers et al., 2008)
	KZ144/ϕKZ	G–	Activity on permeabilized bacteria	(Briers et al., 2007) (Briers et al., 2008)
	OBPgp279/OBP	G–	Activity on intact bacteria	(Walmagh et al., 2012)
	Art-175	G–	Activity on intact bacteria	(Briers et al., 2014) (Defraine et al., 2016)
	LysPA26/JD010	G–	Activity on intact bacteria, Biofilm	(Guo et al., 2017)

Species	Lysin/Phage	Susceptible bacteria tested	Methodology used	References
A. baumannii	LysAB2/ΦAB2	G– and S. aureus	Activity on intact bacteria in vivo: sepsis (mice)	(Lai et al., 2011) (Peng et al., 2017)
	LysABP-01/ØABP-01	G–	Activity on intact bacteria; synergy with colistin	(Thummeepak et al., 2016)
	PlyAB1/Abp1	A. baumannii	Activity on intact bacteria	(Huang et al., 2014)
	PlyF307/RL-2015	A. baumannii; otros G–	Activity on intact bacteria, biofilm in vivo: sepsis (mice)	(Lood et al., 2015), (Thandar et al., 2016)
	LysAB3/A. baumannii ATCC 17978 prophage	A. baumannii	Activity on intact bacteria	(Lai et al., 2013)
	LysAB4/A. baumannii ATCC 17978 prophage	A. baumannii	Activity on intact bacteria	(Lai et al., 2013)

Species	Lysin/Phage	Susceptible bacteria tested	Methodology used	References
E. coli	Lysep3/Ep3	E. coli, P. aeruginosa	Activity on permeabilized bacteria	(Lv et al., 2015)
	Lysep3-D8	G–, Streptococcus sp	Activity on intact bacteria	(Wang et al., 2017)
	Colicin-lysep3	E. coli	Activity on intact bacteria in vivo: intestinal infection	(Yan et al., 2017)
	EndoT5/T5	E. coli	Activity on permeabilized bacteria	(Shavrina et al., 2016)
	PlyE146/E. coli 8.0569 prophage	G–	Activity on intact bacteria	(Larpin et al., 2018)
K. pneumoniae	K11gp3.5/K11	G–	Activity on permeabilized bacteria	(Walmagh et al., 2013)
	KP32gp15/KP32	G–	Activity on permeabilized bacteria	(Walmagh et al., 2013)
	KP27 lysin/KP27	G–	Activity on permeabilized bacteria; cell culture	(Maciejewska et al., 2017)
C. freundii	CfP1 lysin/CfP1	Citrobacter sp.	Activity on intact bacteria	(Oliveira et al., 2016)
S. maltophilia	P28	G– and some G+	Activity on intact bacteria	(Dong et al., 2015)
Burkholderia sp.	AP3gp15/AP3	G–	Activity on permeabilized bacteria	(Maciejewska et al., 2017)

Table 3: Active lysins against Gram-negative pathogens.

Chapter 5 Conclusion

Patients, healthcare institutions, and the global economy are all seriously threatened by antimicrobial resistance. Antibiotic resistance is becoming more severe as new resistance mechanisms arise. To deal with this circumstance, it is essential to identify other alternatives. The bacteriophage-produced enzymes called lysin might be a possible alternative to antibiotics in the fight against rising antibiotic resistance. In this review article, we made an effort to show how lysin can serve as a therapy for diseases that are immune to antibiotics. Throughout the research, there have been a few promising results in the fight against antibiotic-resistant infections. Lysins can selectively destroy infections on mucous membranes without harming the natural flora in the area. This lowers the population's overall pathogen reservoir. It's possible that this feature won't be immediately accepted since it wasn't previously accessible. However, much like with vaccinations, we should be working to create techniques that fight against illness rather than cure it. Anywhere where killing bacteria is required and contact with the organism is permitted, lysins may be widely used. Such enzymes will be useful in hospitals, daycares, and healthcare facilities where antibiotic-resistant bacteria are a problem. The lysins identified so far are thermostable (up to 60°C) and easy to produce in large scale. Protein engineering, domain shifting, and gene shuffling are three methods that could be used to develop improved lytic enzymes that could be used to treat bacterial infections under a variety of situations. Given that there are expected to be 10^{31} phage on the planet, there is a tremendous chance that novel lytic enzymes and those that destroy resistant strains of bacteria can be discovered. By doing this, phage lytic enzymes will play a crucial role in our arsenal against harmful bacteria in the future. Lysins can be a useful stand-in treatment if they are used appropriately. In this area, further study is still required.

Chapter 6 References

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