BACTERIOPHAGE THERAPY AGAINST NOSOCOMIAL INFECTION AND AIRBORNE INFECTION CONTROL IN ICU

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

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Declaration

It is hereby declared that:

1. The thesis submitted is my 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. I have acknowledged all main sources of help.

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Approval

The project titled "**Bacteriophage Therapy against Nosocomial Infection and Airborne Infection Control in ICU**" submitted by Fatema Afreen (19146042) of Spring, 2023 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

This study does not involve any kind of human or animal trial.

Abstract

The spread of antibiotic resistant nosocomial infections and cross transmission of airborne nosocomial infection have urged the scientific community to hunt for prevention and alternative treatments of it. This study aimed to focus on efficacy and safety of bacteriophage as an alternative therapy as well as controlling airborne nosocomial infection by introducing ICU design with well-planned air ventilation system with relative pressure gradients. This was conducted by reviewing a range of relevant literatures and following international health facility guidelines. According to the findings, bacteriophage therapy achieved positive outcomes for the management of nosocomial infections apart from some limitations and well ventilated positive and negative pressurized ICU is indeed a successful technique for limiting airborne infections. Well-planned ventilation systems in ICUs is a public right. More studies and clinical trials need to conduct on bacteriophage therapy to increase the treatment available to the public.

Keywords

Nosocomial infection, Antibiotic resistance, Bacteriophage therapy, phage, airborne nosocomial infection, Positive pressure room, Negative pressure room, ICU.

Dedication

Dedicated to my Beloved Parents

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List of Acronyms

HCAI	Health care associated infection
ICU	Intensive care unit
WHO	World Health Organization
NI	Nosocomial infection
MDRO	Multidrug-resistant organism
MRSA	Methicillin-resistant Staphylococcus aureus
CDC	Center for Disease Control
CLABSI	Central line-associated bloodstream infection
CAUTI	Catheter-Associated Urinary tract Infection
HCAP	Health care–associated pneumonia
SSI	Surgical Site Infection
VRE	Vancomycin-resistant enterococci
RBP	Receptor-binding protein
LPS	Lipopolysaccharides
SPL	Staphylococcal phage lysate
PFU	Plaque-forming unit
PT	Phage therapy
PAS	Patient Administration Systems
HVAC	Heating, ventilation, and air conditioning
ACH	Air change per hour
AHU	Air handling unit
OA	Outdoor air
SA	Supply air
RA	Return air
EA	Exhaust air

Chapter 1: Introduction

Nosocomial infections usually referred to as Health care associated infections (HCAI), develop in a patient receiving medical treatment in a hospital or other healthcare setting that was not present at the time of admission. (Khan & Biag, 2017) This is the sort of infection induced by an extended hospitalization, and it is a significant risk factor for life-threatening health complications. (Khan, 2015) The contemporary hospital is a broad and sophisticated institution that offers a range of diagnostic and curative services towards numerous patients, but the hospital setting has become highly contaminated with various kinds of pathogens and serves as a reservoir of nosocomial pathogens which might infect patients throughout their hospitalization. (Nyamogoba & Obala, 2002) They impact 1 in 10 hospitalized patients, resulting in 5000 fatalities a year and a billion-pound financial burden on the National Health Service. In developing nations, the impact of these illnesses is around 75% higher. (Khan, 2015) Nosocomial infections include those acquired by hospital employees, visitors, or healthcare professionals; invasive equipment such as catheters and ventilators; and infectious airborne particles transmitted from patient to patient. (Khan & Biag, 2017)

The issue of nosocomial infection has existed since the 18th century, and hospitals are regarded as hazardous because of the presence of potentially infectious pathogens in the surrounding environment. (Nyamogoba & Obala, 2002) During the antiseptic and antibiotic era, the issue of nosocomial infection seemed to be resolved. (Mbim, 2016) According to studies, nosocomial infections are on the rise in the twenty-first century (21st century), which is due to the growing use of antibiotics in hospitals that has resulted in the development of resistance in most bacteria, along with certain medical treatments, particularly those that circumvent the body's natural barrier protection, cross contamination from medical personnel to patients or patient to patient through airborne transmission as well as insufficient cleaning practices including uniforms, washing, equipment disinfection, and other preventative steps that may be overlooked, hence allowing germs to proliferate. (Mbim, 2016)

Hospital-acquired infections existed prior to the establishment of hospitals and turned into a public health concern during the miraculous age of antibiotics. (Khan et al., 2015) Antibiotics ushered in a new age in medicine, becoming a vital medical treatment with 262.5 million treatment courses administered in the United States alone in 2011 (842 prescriptions per 1000

persons).(Lin et al., 2017) Since the introduction of antibiotics five decades ago, the evolution of resistance to antimicrobial drugs has been a continuous and dynamic process. (Flaherty & Weinstein, 2015) The concern of bringing contemporary medicine back to a pre-antibiotic era-like condition is threatened by the global proliferation of pathogenic bacteria that are resistant to a range of medicines.(Matsuzaki et al., 2005) Even if novel antibiotics aimed at such drug-resistant bacteria may be generated via research, the infections will ultimately acquire resistance to these drugs.(Matsuzaki et al., 2005) Antibiotic resistance is on the rise, which poses a significant problem when selecting antibiotic treatment for critically ill patients in the intensive care unit (ICU). (Flaherty & Weinstein, 2015) As we are moving towards a post-antibiotic age, the rise and spread of antimicrobial resistance among pathogenic microbes has urged the scientific community and medical professionals to hunt for alternative treatments as the world struggles with an ever-growing issue with antibiotic-resistant bacteria. (Bragg., 2014, Kaur, 2021) In 2017, the World Health Organization (WHO) underlined the threat posed by Gram-negative bacteria resistant to numerous antibiotics (WHO, 2017), thus it is essential to discover, design, and develop new and alternative antibacterial medicines. (Kortright & Chan, 2019) It has been shown that inappropriate therapy, especially treatment to which a pathogen is resistant, is a distinct risk factor for higher mortality in patients with various types of nosocomial infections. (Flaherty & Weinstein, 2015) In the search for alternate treatments for bacterial infection prevention and control, one of the most prevalent ideas is to revisit the practice of Bacteriophage therapy. (Lin et al., 2017)

Bacteriophage is an effective therapy due to its lack of toxic effects on hosts, with the exception of rare, reversible allergic responses, and its high success rate has been reported (Bragg et al., 2014). Félix d'Herelle introduced phage therapy, a technique employing phages to treat bacterial infectious disorders, about 20 years before the commercial use of penicillin, the first antibiotic (Matsuzaki, 2005). During 1910–1917, the first bacteriophage treatment or phage therapy was used to treat bacterial illness, and it was commercialized in the 1940s. However, with the discovery of antibiotics, the majority of nations have ceased using bacteriophage as a therapy (Kaur, 2021). Bacteriophages are extremely host-specific and target a particular strain based on the phage employed which promote the bacterial adherence (Bragg, 2014). According to study reports, appropriate therapy of living phages has the potential to cure fatal infectious diseases induced by gram-negative bacteria, such as *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, Vibrio vulnificus and Salmonella spp*.,

and gram-positive bacteria, such as *Bacillus anthracis*, *Streptococcus pyogenes, S. pneumoniae* and group B *streptococcus.* (Matsuzaki, 2005) Phage treatment has numerous benefits, including the following: (1) it is indeed efficacious against multidrug-resistant bacteria since the processes by which it promotes bacteriolysis vary totally from antibiotics; (2) substituted microbes does not arise as it has great selectivity for target bacteria; (3) it can adapt quickly to the development of phage-resistant mutants since the phages are capable of mutating; and (4) Developing a phage system is less expensive than designing a novel antibiotic and (5) Phage therapy do not harm eukaryotic cells, therefore phages themselves seldom produce side effects (Matsuzaki et al., 2005). Although there is still plenty to study about the interactions between bacteria, phage, and human hosts, it appears that phage therapy is reaching its critical stage (Lin et al., 2017).

Patients in intensive care units are more prone to nosocomial infections. (Fridkin & Welbel, 2005) Since the 1980s, experts in infectious diseases have understood that ICU patients are substantially more likely than other hospital patients to get nosocomial infections. The probability is up to five to ten times higher for ICU patients than it is for those on general medical wards. (Weber & David J., 2015) There are three main variables that contribute to the increased risk of nosocomial infections are the following: (1) extremes of age, malnutrition, several infections, a serious underlying condition, and immunosuppression.; (2) Invasive medical devices, such as, intravascular catheters, endotracheal tubes for ventilation and urinary tract catheters, and (3) crowding (such as, neonatal intensive care units) and animate reservoirs (such as colonized or infected patients), which increase the chance of cross-infection via airborne transmission in the ICU. (Weber & David J., 2015) infected patients, medical staffs, and attendees, infectious particles and droplets, poor ventilation systems within the ICU ward, are all potential sources of airborne nosocomial infection. (Eickhoff, 2015) While breathing, sneezing, or coughing, numerous particles between 100 nm and 1 mm in diameter are expelled at rates between 1 and 10 m/s, some of which are microscopic enough to be thoroughly inhaled in the human respiratory system. They are especially dangerous since they may migrate up to 10 meters inside. (Crawford et al., 2021) By providing clean air and dilution of polluted air using precision designed ventilation systems, aerosol transmission may be effectively controlled and the likelihood of indoor infection reduced. (Izadyar & Miller, 2022) This may be done by regulating the amount and quality of intake or exhaust air, maintaining different air pressures between adjacent spaces, developing airflow patterns for certain therapeutic

processes, utilizing HEPA filters to dilute infectious particles with huge air volumes and other methods (IHFG, 2022).

In order to reduce the risk of nosocomial infection that is airborne transferred, ventilation in the majority of the ICU must ensure a comfortable and secure environment for patients and medical staff. Nevertheless, from the standpoint of infection control measures, the availability of isolation facilities, including negative and positive pressurized room, is the most important feature of proper ventilation in the ICU (N.H O'Connell & H Humphreys, 2002). Positive room pressure, Class P, which protects against airborne transmission of any infection for immune-compromised patients, class N negative pressure rooms protect others from any airborne transmission from a patient who provides an infection risk, and negative room air pressure, Class Q for quarantine isolation, with additional barriers like an anteroom, are suggested for an ICU layout with controlled airflow and relative pressure gradients. (IHFG, 2022) To prevent the spread of airborne infectious particles and reduce the risk of infection, an air ventilation strategy should be combined with an appropriate design of features including supplied-air velocity, controlled airflow, relative pressure gradients, and placement of intake and exhaust. (Izadyar & Miller, 2022)

1.2 Aim of the study

This review aimed at the therapeutic use of bacteriophage as alternatives to antibiotics as well as reducing cross-infection of other persons inside the critical care unit.

1.3 Objectives of the study

The objectives of this review were:

i) to develop protection against multidrug-resistant pathogenic bacteria with distinct advantages for bacteriophage treatment,

ii) to introduce well planned air ventilation system with regulated airflow and relative pressure gradients inside ICU design.

Chapter 2: Methodology

The most recent relevant literatures were thoroughly examined. This review is based on the design of ICU air ventilation system and bacteriophage therapy against nosocomial infection, and it was conducted out by reviewing a range of academic publications, research papers, and related websites, including Pubmed, Elsevier, Frontiers, Nature, ScienceDirect, Springer etc. Basic study was first conducted on antibiotic resistance, bacteriophage therapy and ICU design with a well-planned air ventilation system and a preliminary list of research papers was developed. ICU air ventilation system and pressurized room design suggestions were developed in accordance with international health facility guidelines. The review is afterward conducted out systematically by combining all the considerations. Keywords including Nosocomial Infection, Bacteriophage treatment, antibiotic resistance, multidrug resistance, Ventilation system, ICU layout, etc. were used to search the papers for the review.

Chapter 3

Nosocomial infection

Nosocomial infection (NI), which is defined as an infection that was neither existence nor latent at the time of admission and is associated with higher rates of mortality and morbidity in critical care units (ICU) (Bergogne-Bérézin,1999). The most frequent catastrophic effect in healthcare that compromises safety and quality of patient's care is nosocomial infection (Sikora and Zahra, 2022). The increasing incidence of multidrug-resistant organisms (MDROs), which can effectively make certain categories of antibiotics impotent in treating several frequently reported infectious diseases, is a key contributor in increasing death rates of nosocomial infections. Every year, over 2 million people suffer from antibiotic-resistant infection, and 23,000 of them pass away (Khan and Biag, 2022). As a result, it is suspected that the advent of multidrug resistant organisms would increase the number of challenges related to nosocomial infection, which impacts 6.5% of total of patients in the European Union/European Economic Area and 3.2% of all inpatient patients in the United States (Sikora and Zahra, 2022). Additionally, it has been recognized that the advent of opportunistic new infections with novel resistance issues has contributed to the alteration of nosocomial infection patterns (Bergogne-Bérézin,1999).

3.1 Types of nosocomial infections

Nosocomial infections are commonly categorized into 13 subtypes based on the site of infection, which are determined by both biological and clinical factors, as reported by the National Healthcare Safety Network with the Center for Disease Control (CDC) (Nimer, 2022). Numerous different types of nosocomial infections have been reported, such as legionnaires' pulmonary aspergillosis, Mycobacterium tuberculosis, urinary tract infections, respiratory tract infections, nosocomial fungal infections, bloodstream infections and central nervous system infections, bacterial and viral nosocomial pneumonia (Bergogne-Bérézin,1999). In acute hospital settings, pneumonia is the most frequent HAI, followed by gastrointestinal infections, SSI, bloodstream infections, and urinary tract infections, according to a point-prevalence survey performed in the United States in 2015 (Sikora and Zahra, 2022). The section below includes the typical infection types:

3.1.1 Intravascular catheter infections

One of the most prevalent and fatal nosocomial diseases is central line-associated bloodstream infection (CLABSI) (Bearman and Munro, 2006). Catheters are inserted into the patient's central veins throughout treatment interventions to deliver medications and other supplies (Nimer, 2022). However, continued use might result in severe bloodstream infections, which would threaten health (Bearman and Munro, 2006). The significant proportion of primary bacteremia are associated with intravascular catheterization, which is the greatest distinct risk factor for nosocomial bacteremia (Bearman and Munro, 2006). In the US, CVC affects 24% of non-ICU patients and 55% of patients in intensive care units (Sikora and Zahra, 2022). *Candida* species (13%), *S. aureus* (23%), coagulase-negative *Staphylococcus* (12%), *Streptococcus* species (12%), *E. coli* (8%), *Enterococcus species* (12%), and *Bacteroides* species (6%), based on a recent study conducted in the United States, are the prevalent organisms linked to CLABSI (Sikora and Zahra, 2022). Therefore, prevention methods for these frequent and expensive infection are crucial.

3.1.2 Catheter-Associated Urinary tract Infection (CAUTI)

With a high incidence worldwide, CAUTI is sometimes referred to as one of the most frequently recognized forms of nosocomial infections (Nimer, 2022). The high percentage of urinary tract infections linked with catheters are caused by the patient's own periurethral and perineal flora or by cross-transmission through the use of contaminated hands by medical personnel during the placement and management of the urinary catheter system (Bearman and Munro, 2006). Indwelling urinary catheters are implanted in 15% to 25% of hospitalized patients, and many of them are frequently put in incorrectly (Sikora and Zahra, 2022). After 1 month, 100% of catheters are colonized with a biofilm, where bacteria can grow in a privileged condition from antimicrobial drugs and human defenses, increasing the risk of bacteriuria by 3% to 10% every day (Khan and Biag, 2022). The best suggestion is to prevent urinary catheterization wherever possible and, if necessary, to systematically and pro-actively withdraw catheters as quickly as they are no longer needed (Nimer, 2022). According to several researches, *Enterococcus* species, *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Candida* species are the most prevalent CAUTI pathogens (Sikora and Zahra, 2022).

3.1.3 Health care–associated pneumonias (HCAPs)

The second most prevalent and deadliest nosocomial infection is health care-associated pneumonia (HCAP) (Khan and Biag, 2022). Nosocomial pneumonia typically develops 48

hours after tracheal intubation, and 86% of cases are related to ventilation (Nyamogobe and Obala, 2022). Mechanical ventilation, the clinical setting, indoor environmental quality, a lack of provider infection prevention, cross-transmission, etc. seem to be the sources of HCAPs (Mbim and Mboto, 2016). Recent antibiotic usage and hospital admittance are additional risk factors for HCAPs with antibiotic-resistant pathogens (Bearman and Munro, 2006). The greatest fatality rates and lengthiest hospital stays are seen by individuals with pneumonia brought on by *Pseudomonas* or *Acinetobacter* (Bearman and Munro, 2006).

3.1.4 Surgical Site Infections (SSIs)

The most prevalent unintentional reason for postoperative rehospitalization and the most frequent NI among post - operative patients are nosocomial SSIs (NSSIs) (Nimer, 2022). Generally speaking, 2% to 5% of individuals who undergo surgery have surgical site infections (Nimer, 2022). SSI often happens within the first 30-day period after surgery or 90 days when prosthetic devices are included (Sikora and Zahra, 2022). *Staphylococcus aureus* is the major contributing factor behind this kind of nosocomial infection, which increases the likelihood of mortality and necessitates a lengthy hospital stay. The incidence might reach 20%, depending on the method and monitoring settings applied (Kanouff and Kaplan, 2008).

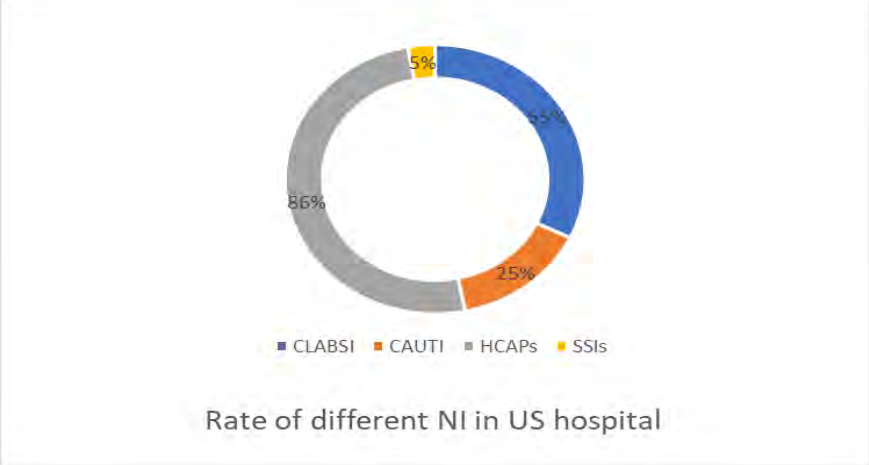

Fig 1: The pie chart indicates the approximate percentage of nosocomial infection (NI) in US hospitals in 2015-2017. (Khan and Biag, 2022)

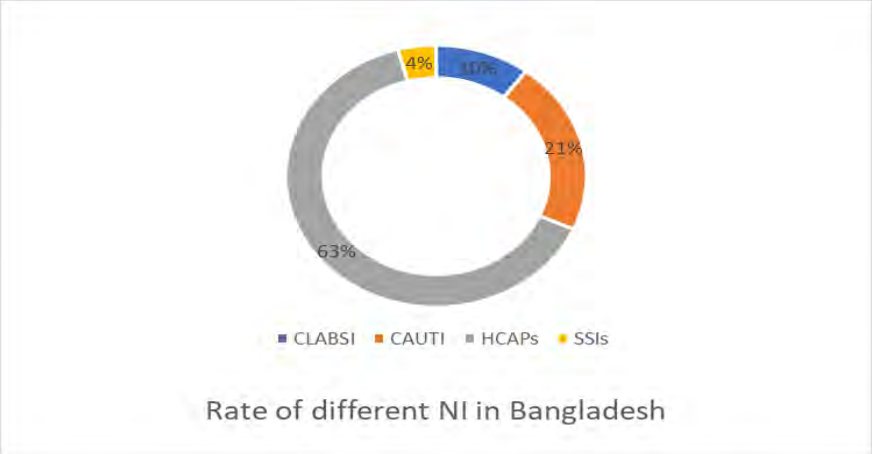

Fig 2: The pie chart indicates the approximate percentage of nosocomial infection (NI) in a Private Hospital of Bangladesh from January 2014 to June 2014. (Begum and Bari, 2017)

3.2 Nosocomial Pathogen

In hospital settings, bacteria, viruses, and fungus parasites are the pathogens that cause nosocomial infections (Nyamogobe and Obala, 2022). Approximately 90% of nosocomial infections are generated by bacteria, with minor contribution occurring from protozoans, fungi, viruses, and mycobacteria (Bergogne-Bérézin,1999). Several bacteria, including *Staphylococcus* spp., *S. aureus, Streptococcus* spp, *Acinetobacter* spp, *Bacillus cereus*, *Escherichia coli*, *Enterococcus* spp., and *Pseudomonas aeruginosa* are prevalent nosocomial infection-causing agent followed by *Proteus mirabilis, Salmonella spp., Serratia marcescens*, and *Klebsiella pneumonia*. (Darvishi and Forootan, 2020) Gram-positive aerobic bacteria with the largest prevalence were *Enterococcus* species, followed by *Streptococcus* and *Staphylococcus* species and *E. coli, P. aeruginosa*, *K. pneumoniae,* and *Enterobacter cloacae* were the most widespread categories of the Gram-negative aerobic bacteria. (Bergogne-Bérézin,1999) Leading nosocomial pathogens by site and frequency are shown below:

Table 1: Prevalence of nosocomial pathogens responsible for different types of nosocomial infection.

Infection site	Pathogen	Frequency	Reference
Intravascular catheter infections	*S. aureus*	23%	(Bergogne-Bérézin, 1999), (Sikora and Zahra, 2022)
	coagulase-negative staphylococci	12%	
	Candida spp	13%	
	Enterococcus spp	12%	
	E.coli	8%	
	Streptococcus spp.	12%	
	Bacteroides spp.	6%	
Catheter-Associated Urinary tract Infection	*Escherichia coli*	24%	(Flores-Mireles and Hreha, 2019), (Bergogne-Bérézin, 1999), (Sikora and Zahra, 2022)
	Candida spp.	17.8%	
	Enterococci	14%	
	P. aeruginosa	10.1%	
	Enterobacter spp.	10.3%	
Health care–associated pneumonias	*P. aeruginosa*	15.7%	(Suljevic and Asotic, 2020), (Bergogne-Bérézin, 1999), (Sikora and Zahra, 2022)
	S. aureus	11.8%	
	Klebsiella pneumoniae	7.8%	
	Acinetobacter baumanni	43.1%	
	Enterobacter spp.	3.5%	
Surgical Site Infections	*Enterococcus*	5.9%	(Birhanu and Endalamaw, 2020), (Bergogne-Bérézin, 1999), (Sikora and Zahra, 2022)
	Enterobacter spp.	4%	
	K. pneumoniae	4%	
	coagulase-Negative staphylococci	11.7%	
	S. aureus	30.4%	

	P. aeruginosa	5.5%	

3.2.1 Antibiotic-resistant nosocomial pathogens

Antibiotic resistance is more prominent in organisms obtained from intensive-care unit patients in comparison to organisms obtained from general-ward patients or outpatients (Vincent, 2003). Antibiotics are readily accessible at pharmacies in developing nations, and the excess use of a variety of antibiotics in hospitals has resulted in the introduction of MDR bacteria, which are lethal nosocomial infections that are challenging to cure (Darvishi and Forootan, 2020). MRSA, VRE, third-generation cephalosporin-resistant *Enterobacteriaceae*, imipenem-resistant *P. aeruginosa*, and *A. baumannii* are some of the resistant bacteria that are of major concern due to their global emergence and potential negative effects on the management of infections brought on by these organisms (Kumar and Tanwar, 2022). Over the period of four years (2014–2011), it was discovered that the average rates of *E. coli* resistance to third-generation cephalosporins (from 9.6 to 12%), *K. pneumonia* resistance to carbapenems (from 6.6 to 7.3), *P. aeruginosa* resistance to carbapenems (from 16.8 to 18.3%), and *Enterococci* resistance to vancomycin (from 6.2 to 7.9) had all significantly increased throughout Europe (Darvishi and Forootan, 2020). In the United States, resistant microorganisms account for between 50 and 60 percent of hospital-acquired infections, and incorrect administration of antibiotics is regarded to be the main contributor to this drug resistance (Weber and David, 2015). Antibiotic resistance for selected nosocomial pathogens are listed below:

Table 2: Multidrug resistance in prevalent nosocomial pathogens.

Organisms	Key antibiotic resistance	Reference
Gram-positive organisms		(Darvishi and Forootan, 2020), (Kumar and Tanwar, 2022), (Bergogne-Bérézin,1999), (Vincent, 2003)
Staphylococcus aureus	Penicillin-resistant; methicillin-resistant (>90%); Cefoxitin, Azithromycin (76%) Cefotaxime (80%), Tetracycline (75%), Gentamicin (80%; fluoroquinolones carbapenems.	
Coagulase-negative *Staphylococci*	Methicillin resistant: (>70%); clindamycin, tetracyclines, cotrimoxazole, Quinolones (65%),Vancomycin	
Enterococcus spp.	Vancomycin (35.5%), ampicillin (<80%), cephalosporins, fluoroquinolones, doxycycline, clindamycin	
Gram-negative organisms		
Pseudomonas aeruginosa	Third-gen cephalosporins, imipenem, doxycycline, cotrimoxazole,quinolones, gentamicin, tigecycline. Colistin (13.3%),carbapenem groups (16.6%), tazobactam (91%), ceftazidime (93.3%), cefoperazone (100%)	
Klebsiella pneumoniae	β-lactamase antibiotics such as ampicillin, cephalosporins, ceftriaxone or ceftazidime (75%), amikacin (64%), amoxiclav (100%), ceftazidime (88%), cefoperazone (92%), doxycycline (100%), ciprofloxacin (60%), ofloxacin (64%), and tazobactam (80%).	
Acinetobacter baumannii	Fluoroquinolones (92–100%), cephalosporins (87.5–96%), tazobactam (92%), imipenem (71%) , doripenem (79%), and tigecycline (62.5%).	
Enterobacter spp.	Aztreonam, amikacin, fluoroquinolones,cephalosporins, ceftazidime, third gen cephalosporins, Imipenem	

Chapter 4: Treatment

4.1 Phage Therapy: A Renewed Approach

By 2050, it is anticipated that 10 million deaths per year might be caused by the serious public health issue of antibiotic resistance (Oechslin, 2018). Antibiotic resistance was the topic of discussion at the United Nations General Assembly on September 21, 2016, and it was declared "the greatest and most urgent global risk" (Broncano-Lavado, 2021). Despite the fact that new medicines targeted against these drug-resistant bacteria may be developed when enough research funding is devoted, the pathogens eventually acquire resistance to such medications (Hasan & Ahn, 2022). In the search for alternate techniques for bacterial infection prevention and control, one of the most prevalent recommendations is to re-evaluate the concept of phage treatment (Broncano-Lavado, 2021). Nevertheless, difficult problems still persist, such as choosing the most effective phage (or phages) to combat a specific infection, the danger of phage resistance development, the host's immunological reaction to phages, as well as unique regulatory needs (Oechslin, 2018).

Bacteriophages are quite specific infectious viral particles that, when they adhere to cell surface receptors during the lytic cycle, rapidly enter and destroy the bacterial cell. As a result of the production of newly infectious virions throughout the lytic cycle, the target bacterial population may be suppressed by further attacking of surrounding bacterial cells by those new virions. (Matsuzaki, 2005). Infections that are challenging to cure may be treated with them alone or in conjunction with antibiotics (Oechslin, 2018). Phage treatment has a variety of unique benefits, such as the possibility of working with a single dosage since pathogenic phages may multiply until host bacterial cells are available, which therefore leads to auto-dosing (Matsuzaki, 2005). It has been found by studies that Bacteriophages may be discovered in almost all organs when given intravenously, making them the perfect choice for treating localized infections in various regions of the body. This is a benefit compared to other antibiotics that phage therapy only targets particular organs during therapy (Cisek & Dąbrowska, 2016). Furthermore, since they only multiply in the presence of the bacteria that are causing the disease, they exhibit great host specificity and are safe for eukaryotic cells, generating no negative side effects and lessening harm to the environment's normal microflora, therefore, It is considered to be a safe treatment for bacterial infection (Aranaga & Pantoja, 2022). Osteomyelitis, sepsis, bacteremia, otitis media, infections of the urinary tract, lungs, and infections caused by prosthetic devices have

all been treated using phage therapy. Phage therapy has also been used to treat infections caused by burn injuries, soft tissue damage, burns, and sepsis. In cases when a patient has to stay at hospital for a prolonged time and many antibiotics have been attempted without success in curing an infection, phage therapy can sometimes give better results (Aranaga & Pantoja, 2022).

Research on phage treatment has not been very thorough. In April 2021, a PubMed search for "phage treatment" returned 1,664 results, the vast majority of which (96.69%) were published during the last decade. This discipline has struggled for years with a severe dearth of information about several topics, including insufficient understanding of tolerance, immune response, pharmacokinetics, pharmacodynamics, and appropriate research involving animal infection models. Recent years have seen a revival in phage treatment research in Western nations like France, Belgium, the Netherlands, the United Kingdom, Switzerland, and the USA, among others. Additionally, phage treatment was included as one of the seven approaches by the US National Institute of Allergy and Infectious Diseases in 2014 to combat antibiotic resistance (Broncano-Lavado , 2021, Reardon, 2014).

4.1.1 History: Early studies of phage therapy

The concept that they might be applied clinically to combat bacterial infections arose from their capacity to destroy bacteria with efficiency. This method, termed as phage therapy, has a lengthy history (R Clark, 2015). Despite the fact that the concept of employing bactericidal viruses therapeutically to treat bacterial infections has only gradually acquired popularity in reaction to the appearance of multidrug-resistant bacteria, the technique has been in existence for about a century (Fridkin & Welbel, 2005).

Two microbiologists, Frederik Twort in 1915 and Félix d'Hérelle in 1917, each worked independently to make the discovery of phage. Although Twort was the first person to detect and demonstrate the impacts of a "transparent material" that prevented bacterial development, it wasn't until 1917 that d'Hérelle identified an anti-Shigella microbe that the concept of an obligatory parasite of bacteria was referred to as a "bacteria-eater" or "bacteriophage" (Lin & Koskella, 2017). After proving the phage therapeutics preliminary safety by applying the therapy on himself and further on his coworkers, family, patients with "bacillary dysentery" and cholera (since 1919), d'Herelle started evaluating it on human patients with serious dysentery at the Hospital des Enfants-Malades in Paris in 1917 (Nikolich & Filippov, 2020). The scientists reported that the infections subsided within 24 to 48 hours after injecting bacteriophages into and surrounding surgically opened wounds in 1921 (Sulakvelidze, 2001).

In the Punjab area of India, d'Herelle saw a 90% decrease in death rates in the investigational group during a 1931 trial of phage therapy as a cholera treatment. In addition to d'Herelle, numerous other entrepreneurs sought to market phage manufacturing; but, owing to the drawbacks of phage treatment and the advent of pharmaceutical antibiotics in the 1940s, phage therapy was largely disregarded by western medicine (Fridkin & Welbel, 2005).

4.1.2 Bacteriophage Classifications and structure

The *Caudovirales* order, which contains the *Myoviridae*, *Podoviridae*, and *Siphoviridae* families of "tailed phages" with an icosahedral head and tail and single- or double-stranded RNA or DNA as their genetic material, accounts for about 96% of all documented phages (Bragg, 2014, Giri, 2021). Tailed phages have an icosahedral head and a lower portion covered with receptor-binding proteins (RBPs) resembling tail spikes and tail fibers (Hasan & Ahn, 2022). The head is joined to the tail by a collar, that might or might not be stretchable, and whose lower portion is in touch with tail fibers whose endings identify points of attachment on receptors on the surface of the bacterium (Wittebole & De Roock, 2013). The stretchable sheath, which acts as a conduit for delivering the genetic material to the bacterial cells, and a distinctive adsorption mechanism that can detect the host cell receptor are the two essential parts of the tail (Giri, 2021). The key emphasis of phage therapy has been on lytic phages, which are primarily found in three families of the *Caudovirales* order: the *Myoviridae*, the *Siphoviridae*, and the *Podoviridae*. In the *Myoviridae* family, RBPs are seen as short and long fibers connected to the contractile tail, in the *Podoviridae* family, RBPs are seen as spikes or fibers linked to a shorter non-contractile tail, and in the *Siphoviridae* family, RBPs are seen as baseplates, fibers, spikes, or singular linear filaments linked to a prolonged non-contractile tail \(Hasan & Ahn, 2022, Oechslin, 2018).

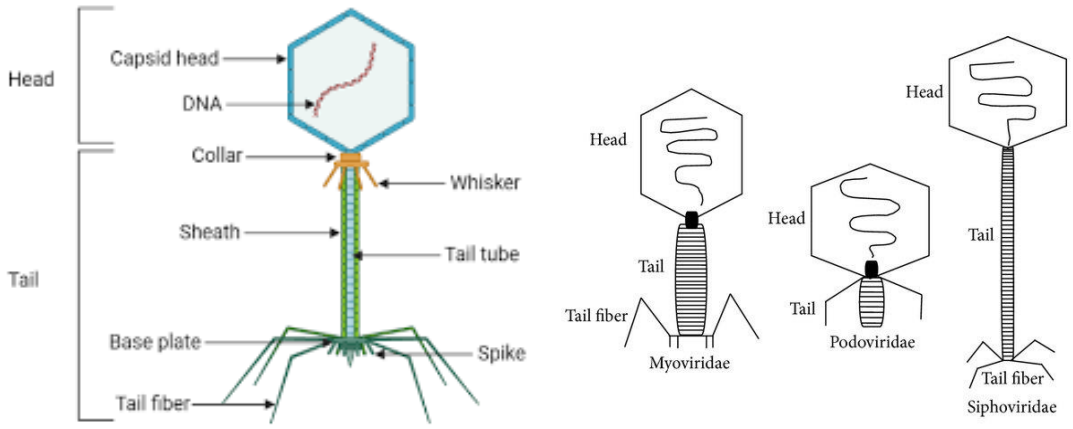

Fig 3: Structure of lytic phages

4.1.3 Life cycle of phages

The ability to proliferate for phages is entirely reliant on their hosts (Hasan & Ahn, 2022). In addition to the morphological categorization scheme, phages may be generally classified into two categories based on their life cycle: "the lytic phage" and "the lysogenic phage" (Fig. 2). (Bragg, 2014). A virus enters the lytic cycle once it seizes control of a host cell, consumes the cell's components to produce more virus, then rips the cell apart, escapes, and infects further cells (Pina & Salinas, 2022). In order to effectively produce several copies of fully developed virus particles and eventually lyse the host cell, virulent phages undergo a lytic life cycle and hijack the host's replication processes (Lin & Koskella, 2017). The lysogenic cycle occurs once a lysogenic phage integrates itself into the genetic material of its infected bacterial host and takes up residence there permanently (Ghannad & Mohammadi, 2012). The genetic material of temperate phages is inserted into the bacterial genome, within which it is replicated as a component of the bacterial genome and stays unnoticed for a long time (Wittebole & Roock, 2013). Through site-specific recombination or transposition, phage DNA is integrated into the bacterial genome and is known as a prophage (Giri, 2021). UV light, antibiotics, pH, temperature, and water activity all work together to cause prophage activation, which results in an irreversible switch from the lysogeny life cycle toward the lytic cycle and the phage then accomplishes the lytic cycle by generating replicates and lysing the cell to blast them out (Hasan & Ahn, 2022). Additionally, they are much more host-specific than virulent phages since they are only capable of infecting the host in which they have been incorporated (Lin & Koskella, 2017). However, to destroy the host cell, the majority of them use two different protein groups. Holins from the first group work in conjunction with endolysins from the second group to produce lysis. Jointly, they constitute the holin–lysin systems (Cisek & Dąbrowska, 2016).

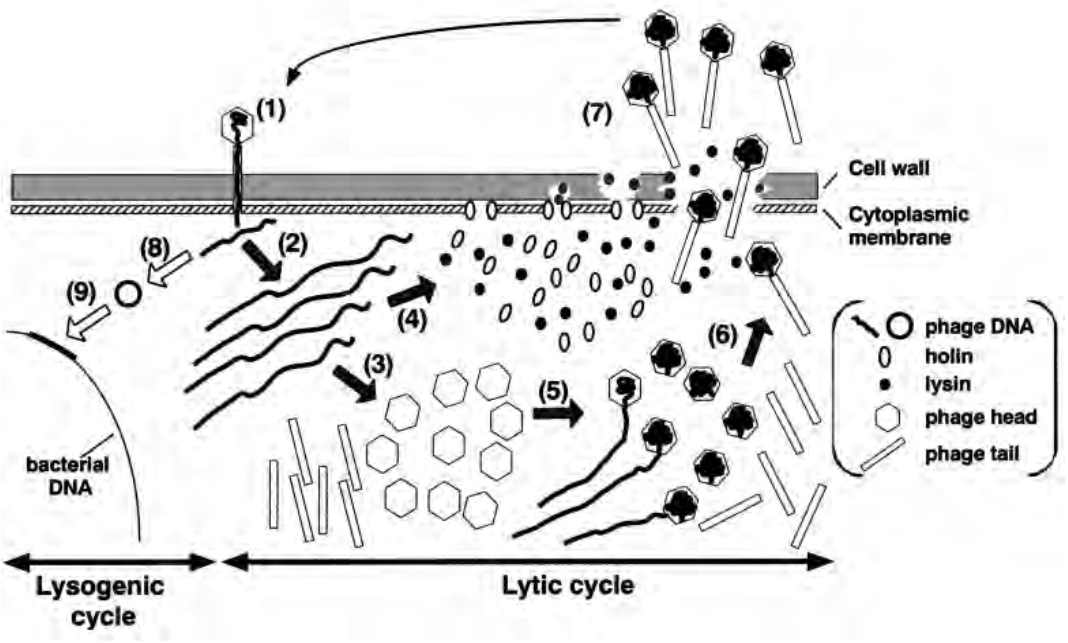

Fig 4: Representation of phage-induced bacteriolysis in a schematic way.

4.2 Bacteriophage as Therapeutic Agent

In recent years, it have seen the beginning of authorized therapeutic usage of phage therapy in various Western European nations (Belgium, France), meanwhile in the US, a number of companies are working on phage therapies to place them into therapeutic usage, however they have not yet received US Food and Drug Administration approval (Nikolich & Filippov, 2020). In the post-antibiotic era, there is great potential for using bacteriophages to cure bacterial infections. Understanding every detail of the phages' biology is crucial in order to adequately assess the promising usage of bacteriophages as a therapy alternative (Lin & Koskella, 2017). In this section, we focus on the phage treatment mechanism of action, phage therapy pharmacokinetics, phage safety, phage effectiveness, phage usage as probiotics, and clinical studies that may evaluate phage therapy based on whether or not the amount of bacteria is decreased or clinical symptoms improve.

4.2.1. Mechanism of action

After entering the body, bacteriophages carry out a process known as lysis (Pina & Salinas, 2022). Adhesion to the receptor, often a protein or sugar on the bacterial cell surface, is the initial stage of the phage lysis process (Fridkin & Welbel, 2005). Therapeutic phages were believed to have a bactericidal effect by proliferating inside and inactivating the host cell

through a lytic cycle (Bragg, 2014). The lysin proteins may enter and hydrolyze the bacterial cell wall at the final stage of the lytic cycle because the holin proteins cause a hole on the cytoplasmic side of the cell membrane (Fridkin & Welbel, 2005). Following the elimination of the bacteria, it will cease growing and stay dormant until new bacteria are ingested by the body. Additionally, phage shows great specificity toward bacteria, which means that they solely fight bacteria, therefore, the phage will not harm human cells (Pina & Salinas, 2022). Compared to bactericidal drugs like vancomycin, oxacillin, and rifampicin, phages appear to have a greater bacteriolytic action (Bragg, 2014).

4.2.2. Specificity

Phages are quite selective to their bacterial host, usually affecting particular strains or subtypes of bacteria (R Clark, 2015). This might be advantageous in certain circumstances since phages can eliminate particular undesirable strains while allowing the remainder of the microflora unharmed. For instance, it has been suggested that phages may be utilized as probiotic supplements, primarily killing on bacteria like Clostridium difficile that produce an imbalance in the gut while preserving the other types of typical gut microflora unaffected (Bragg, 2014). Phage specificity may be quite advantageous in certain circumstances, particularly in view of the well-known negative effects of broad-spectrum antibiotics on many disorders. Host specificity, which restricts the feasibility of large-scale manufacture and dissemination, has a substantial influence on therapy design and evaluation (Fridkin & Welbel, 2005). The restricted lytic spectrum and host range of many phages, which result from their high specificity, prevent them from treating the variety of bacterial pathogens. Additionally, for certain bacterial diseases, multiple phage combinations may be required to cure the same bacterial infection and Finally, bacterial strains may become resistant to phages owing to changes in their genetic makeup (Nikolich & Filippov, 2020). The prevalence of phages for many bacteria, however, could allow the production of cocktails and their usage may also lessen the likelihood of resistance emerging (R Clark, 2015).

4.2.3 Formulation

The objective for researching novel bacteriophage formulations is to enable physicians to administer phages more efficiently at the location of infection and enhance patient health outcomes (Danial & Jason, 2021). When subjected to stressful circumstances, bacteriophages are prone to the consequences of protein aggregation and misfolding as well as denaturation, causing a loss of functioning (Danial & Jason, 2021). Earlier studies have shown that phages

are sensitive to organic solvents, pH, temperature, and salt. Researchers have developed many strategies for the long-term preservation of free phage. The majority of phage formulation approaches depend on some type of encapsulation. This is a wide terminology that refers to a variety of processes, such as emulsification, spray-drying, freeze-drying, liposome encapsulation in which bacteriophages are encased by particular stabilizing chemicals, giving protection from the external conditions (Chang, 2018). Several distribution techniques are available in the case of a localized skin infection. Emulsion encapsulation will enable the development of a topical cream that could be given directly to the site of infection. Phage-coated powders may be produced via lyophilized and spray-drying procedures and then combined into a cream for topical use, pill-form for oral treatment, or an inhaler system. Immobilization might be used to create phage-coated patches for topical application. The original liquid lysates may also be administered in parenteral route or as drops for oral consumption. (Danial & Jason, 2021).

Table 3. Various encapsulation approaches in bacteriophages formulation (Danial & Jason, 2021)

Encapsulation Method	Bacteriophage	Formulation	Observation
Emulsification	K (*Staphylococcus*)	Semi-solid	10 days activity at 20 ° C
Freeze- drying	M13 (*Escherichia*)	Powder	< 1 log drop after 60 days at 20° C
Spray-drying	PEV2, PEV40 (*Pseudomonas*)	Powder	< 1 log drop after 1 year at 20° C
Liposome Entrapment	KP01K2 (*Klebsiella*)	Liquid	In vivo action lasts up to 14 days.

Formulations may enable viruses survive in severe circumstances for prolonged periods of time, allowing for more therapeutic applications. Phages found in dry, non-liquid formulations are often more stable over time, while they are still susceptible to heat and other stressors that might cause a titer decline (Leung, 2017). Furthermore, the particular method used to develop a particular formulation might lead to bacteriophage deterioration, as shown by the freeze-drying and spray-drying technique. All of these variables must be taken into account when establishing developing stable phage formulations, such as focusing on evaluating phage

delivery to the target bacteria, determining the level of stability a formulation offers under various conditions, and enhancing phage survival throughout formulation production (Dini, 2013). A suitable formulation will indeed enable for large-scale manufacture of the product with the understanding that it can be readily preserved with minimum periodical decline in phage (Danial & Jason, 2021).

4.2.4. Safety

Humans that are often exposed to vast quantities of phages as a component of their normal ecology typically tolerate them well (R Clark, 2015). Phages have been used therapeutically for a very long time in Eastern Europe and the former Soviet Union, as well as prior to the advent of antibiotics in the United States. Phages have been utilized to humans orally, rectally, topically, locally, in tampons, as aerosols or intrapleural and intravenous injections, and that almost without any reports of severe complications (Sulakvelidze, 2001). The administration of phages in 153 patients was described in a study, which extensively addressed the safety of phages and concluded that they seem to be harmless (R Clark, 2015). Phage treatment has just lately received interest by western medicine, in comparison to the extensive research on antibiotic safety, and as a consequence, most of the material that is now available regarding phage safety is novel (Fridkin & Welbel, 2005). However, though the immune reactions don't have an impact on the safety of phage therapy, they may have an impact on effectiveness. Phages administered systemically may be eliminated by the immune system before having a therapeutic impact. At that point, topical preparation is often the initial target for phage treatments. According to some studies, phages may be employed systemically, however, if certain circumstances are met, such as, the allergic history or the condition of the patient (R Clark, 2015). Usiak-Szelachowska et al. recently investigated the deactivation of phages by the host immune system reaction and discovered that, after local or local/oral administration, 12.3% of patients exhibited strong serum antibody activity against the phages, while oral application alone did not produce substantial antiphage reactions . High systemic antiphage reactions, the authors point out, do not exclude the effectiveness of phage treatment, especially when applied topically (Bragg, 2014). As the number and types of ways to use phages expands, it may be crucial to exercise caution when choosing phages and putting them to use in therapy to keep this remarkable track record progressing (Sulakvelidze, 2001).

On the contrary hand, bacteriophage treatment may raise some major issues. For instance, certain phages have the capacity to alter host bacteria in a manner that can increase their pathogenicity. In this situation, it is crucial to prevent from utilizing temperate phages for phage

treatment (Kaur, 2021). There are further issues, such as the fact that Tetz and Tetz employed a mouse model to show that oral treatment of a commercial Russian phage cocktail might increase intestinal permeability and elevate serum levels of inflammatory circulating immune complexes in the blood, which have been linked to a variety of clinical problems. However, a different research found that cytokine levels did not significantly rise after phage therapy (Fridkin & Welbel, 2005). However, the physiological reaction to phages probably varies across people and is influenced by the particular phage strains utilized (Fridkin & Welbel, 2005). Lytic phages cause the disintegration of bacteria at the conclusion of their antibacterial activity, freeing different bacterial components like endotoxin (LPS) from gram-negative bacteria. This might explain a number of adverse consequences on the host, including the emergence of an inflammatory reaction that leads to the malfunction of many organs. When managing bacterial infections locally, these problems are less of a worry (Wittebole & De Roock, 2013). The capacity of phages to migrate all through the body and proliferate where necessary may be altered to minimize lysis, which reduces the risk of toxin release. However, this defeats one of the fundamental benefits of phage treatment (T. Abedon & Kuhl, 2011). Clearly, there are still a lot of safety issues with phage treatment that require to be resolved. Future research must concentrate on human clinical trials to evaluate the safety of phage therapies with reference to human health (Fridkin & Welbel, 2005).

4.2.5. Pharmacokinetics

Although phage treatment has been extensively studied, there are relatively few papers that outline the pharmacokinetics of therapeutic phage formulations (Sulakvelidze, 2001). According to the few papers on the issue, phages reach laboratory animals' circulation (after a single oral dosage) within 2 to 4 hours, and they are discovered in their internal organs (liver, spleen, kidney, etc.) after around 10 hours (Bragg, 2014). The durability of injected phages has also been studied, and results suggest that phages may survive in the human body for quite extended periods of time, up to a few days (Sulakvelidze, 2001). But some research findings gave evidence of antipseudomonal phages and their observed pharmacokinetics in the context of overall phage pharmacokinetics. For instance, the pharmacokinetics assessment of antipseudomonal phage PEV20 (in vivo utilizing IV administration in rats) showed that the phage selectively accumulated in the liver and spleen, clarifying a non-homogeneous phage distribution (Holger & Kebriaei, 2021). Generally, the organ wherein active phages may be found for the longest period of time following injection is the spleen. When injected intravenously, lymph nodes, liver, and spleen have been identified as the greatest phage

delivery organs (Holger & Kebriaei, 2021). Furthermore, several studies have used the non-replicating phage as a standard medicine and shown that the non-replicating phage clearance rate lessens with time, indicating that the phage half-life is dose-dependent (Diallo & Dublanchet, 2022).

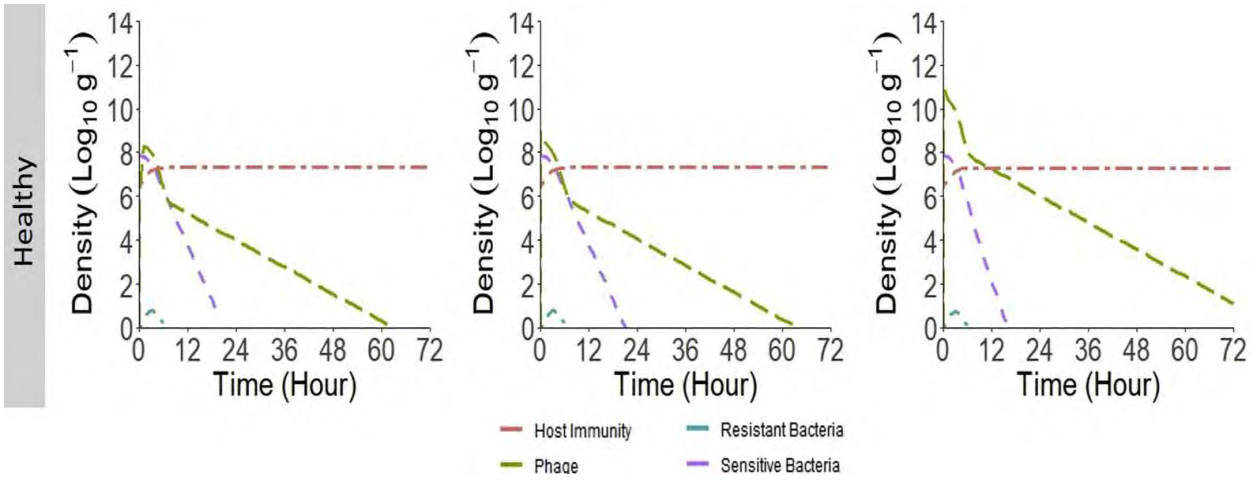

Fig 5: Pharmacokinetic model of antipseudomonal phage ΦPEV20 against ΦPEV20-sensitive bacteria (Lin & Chang, 2020)

4.2.6. Clinical trials for bacteriophage therapy

Thorough studies to confirm safety and effectiveness must be developed in order to move toward clinical usage. The usefulness and safety of bacteriophage treatment against bacterial pathogens such *vancomycin-resistant Enterococcus faecium, Pseudomonas aeruginosa, Clostridium difficile, Staphylococcus aureus,* and *Klebsiella pneumoniae* have typically been validated by animal experimentation (Lu & Koeris, 2011)). Multiple human safety and effectiveness experiments have been conducted due to the growing interest in phage treatment in the West. Staphylococcal phage lysate (SPL) and mixtures that target *P. aeruginosa*, Staphylococcus aureus, and E. coli at concentrations between 10^{5} to $3.6x10^{9}$ plaque-forming units have undergone human safety testing. This research found no increase in unfavorable effects related to the use of phage treatment and phage cocktails. These studies' successes have sparked further investigation into cutting-edge methods for overcoming phage therapy's technical challenges (Broncano-Lavado , 2021).

Table 4: Human safety trial for bacteriophage therapy.

Infection	Treatment group	Phage dose	Outcome	Reference
Pseudomonas aeruginosa (otitis)	12 individuals were given phage cocktail	10^9 PFU single intra-aural dose	At the end of the trial, three people from each group were discovered to have undetectable levels of *P. aeruginosa*; no major side effects were observed in either group, demonstrating the safety of phage treatment.	(Lu & Koeris, 2011), (Broncano-Lavado, 2021), (Wright & Hawkins, 2009)
Escherichia coli (diarrheal diseases)	40 individuals were given phage cocktail M,	1.4 X 10^9 PFU cocktail M administered orally 3 times a day for 4 days in ORS	There was no clear differentiation between the placebo and phage treatment groups; there were also no notable adverse effects.	
Pseudomonas aeruginosa burn wound infection	12 individuals were given a phage cocktail	200– 2,000 PFU topically applied 1 time a day for 7 days	Trial was stopped due to inadequate effectiveness; this may be because a far lower phage dosage was administered than anticipated.	
Escherichia coli; Staphylococcus aureus; Pseudomonas aeruginosa	Intralytix phage cocktail WPP-201 was given on 65 patients	1 X 10^9 Phage cocktail was topically administered to wound infections once a week for 12 weeks.	various rates of wound healing throughout time	

4.2.6. Utilization of phage lysin as therapeutic agent

The discovery of phage-encoded lytic enzymes, which help the phage by hydrolyzing the cell membrane to generate viral reproductive cells, is one of the most promising recent developments in phage treatment. The identification and characterization of these proteins provide an opportunity for the establishment of new phage-based medicines (Fridkin & Welbel, 2005). The majority of tail phages generate endolysin or lysin, a peptidoglycan hydrolase, to discharge their reproductive cells during the last step of proliferation (Bragg, 2014). The bacterial cell wall is hydrolyzed by lysin in two steps: the first stage involves binding to specific locations on the cell wall, and the second involves cleaving the peptidoglycan linkages (Ghannad & Mohammadi, 2012). Each phage may produce a variety of distinct lysins, some of which have a narrow range of action across strains or even between species, as in the case of the newly identified lysin ABgp46, A. baumannii, P. aeruginosa, and Salmonella typhimurium are a few gram-negative and multidrug-resistant bacteria that may be killed by ABgp46. 7 However, Gilmer et al discovery of the very strong lysin PlySs2, which was extremely efficient against a variety of pathogenic Streptococcus and Staphylococcus species, including MRSA, and was completely functioning, showed that not all lysins exhibit equal therapeutic potential (Fridkin & Welbel, 2005). Lysins may even penetrate epithelial cell membranes to cure S. pyogenes intracellular infections, according to a new research investigating the extraction and application of phage proteins (Fridkin & Welbel, 2005). Mice infected with Streptococcus pneumoniae, Bacillus anthracis, Streptococcus pyogenes, and group B streptococcus were used to test the in vivo effectiveness of lysin therapy. Treatment with lysin against systemic infections as well as localized infections in the vagina or nasal cavities has been shown to be beneficial (Bragg, 2014).

There are several benefits to utilizing lysins as an antibacterial therapy agent, including the following: I They are a viable alternative for treating bacteria that are resistant to antibiotics; (ii) Immunogenicity is not a concern for their efficacy; (iii) Appropriate dose of lysin is considerably low (milligrams or even micrograms per litre), so allergic reactions and immune system neutralization are protected; most importantly, resistance to the lysins is rare since the changes modifying the bacterial cell wall would eventually destroy the bacterium and Finally, (v) They quickly reduce the pathogenicity of bacteria (Ghannad & Mohammadi, 2012). Lysins have been evaluated for the management of numerous bacterial diseases, as mentioned above, and they seem to be a handy alternative among antibacterial therapeutic options. However, they are more costly to produce than antibiotics (Ghannad & Mohammadi, 2012 , Bragg, 2014).

4.3. Major limitations of bacteriophage therapy

Considering all the characteristics of lytic phages that would seem to promote their clinical application, they are not often employed globally for prophylaxis or therapy, and their effectiveness is still up for debate. Different factors have led to this circumstance, such as, development of phage resistance, host immune response and so on. (Sulakvelidze & Alavidze, 2001).

4.3.1. Bacterial resistance to bacteriophages

Bacterial resistance to bacteriophages is an important consideration in phage treatment. Similar to how they do with antibiotics, bacterial infections may become resistant to bacteriophages (Broncano-Lavado , 2021). The development of phage resistance in bacteria is a risk similar to that of antibiotic resistance, and it may limit the use of clinical phage treatment (Matsuzaki, 2005). Bacterial resistance to a particular phage may be mediated by at least four different mechanisms. Destruction or absence of receptor, structural change of the binding site, or covering up of the receptor would inhibit phage adhesion to the bacterium and subsequent capacity to produce new phages (Wittebole & De Roock, 2013). The suppression of phage attachment to the host bacterium is crucially dependent on the alteration in the cell surface binding sites, including pili, flagella, outer membrane proteins, and LPS (Hasan & Ahn, 2022). It has been discovered that the E. coli protein TraT alters the shape of Outer-Membrane Protein A (OmpA), the binding site for T-even-like phages. Exopolysaccharide release by Pseudomonas species or glycoconjugate release by Enterobacteriaceae might cover up the binding site, however phages may overcome this by choosing a different receptor or by discharging an exopolysaccharide degrading enzyme (Wittebole & De Roock, 2013). Abortive infection (Abi) systems are another kind of resistance that may prevent the replication, transcription, and translation of phage-infected cells, shielding surrounding unaffected cells from phage attacks (Hasan & Ahn, 2022). During clinical studies on both animals and humans, phage resistance in strains of bacteria has been found (Matsuzaki, 2005).

However, since bacteriophages are thought to have the capacity to evolve biologically alongside their host bacteria, there is significantly less reason to be concerned over bacteriophage resistance than there is regarding antibiotic resistance. Therefore, if a bacterium acquires tolerance to a bacteriophage, the bacteriophages might modify via alteration and evolve to attack the resistant bacterium once again (Lin & Koskella, 2017). Second, due to the

numerous different kinds of bacteriophages found in the environment, there is a good chance that other bacteriophages might still have the capacity to infect a specific bacteria even if it gains tolerance against only one bacteriophage. If a bacteria acquire resistance to antibiotics, there are only a limited number of medicines available, and finding a new antibiotic is not easy (Lin & Koskella, 2017, Matsuzaki, 2005). According to an in vitro research by Oechslin, even if certain mutations give phage resistance, these mutations may reduce bacterial physical ability while compared to standard proliferation under less rigorous in vitro settings in animals. This might reflect the efficacy of PT in certain patients despite the existence of phage-resistant bacteria, demonstrating that a patient's immune system is crucial to the short-, medium-, and long-term effectiveness of PT (Aranaga & Pantoja, 2022).

4.3.1.1. Suggested solution: Phage Cocktail

Phage cocktails may be used to decrease the incidence of bacterial resistance to phages (Matsuzaki, 2005). The primary goals of producing phage cocktails are to increase the possible target pathogen population, expand the phage host range, and enhance efficacy. As a consequence, there is a better chance for empirical therapy (Oechslin, 2018). A cocktail combining phages EFDG1 and EFLK1 was extremely effective against both planktonic and biofilm cultures of vancomycin-resistant Enterococcus, which revealed the development of phage EFDG1-resistant Enterococcus faecalis, which was rapidly and effectively dealt with by isolating a new phage EFLK1 and developing a cocktail containing both phages. Furthermore, utilizing a combination of three phages rather than monotherapy resulted in noticeably reduced rates of phage-resistant K. pneumoniae mutants, according to the researchers. Similar findings were found regarding E. coli, where phage cocktails reduced the incidence of phage-resistance or postponed the formation of phage-resistant varieties (Matsuzaki, 2005). Only pathogenic phages are identified, grown, and generated in bioreactors for the purpose of producing phage cocktails using bacterial strains collected from the patients who are suffering from septic disease. Then, To produce a phage cocktail, the purified bacteriophages are combined in the proper proportions (Matsuzaki, 2005).

Pairings of phages and antibiotics in therapy may provide synergies that need to be utilized to both improve the efficacy of antibiotics and incorporate feasible combination therapies into the bacterial infection; thus, knowing the relationships between phages and antibiotics is a crucial objective (Nikolich & Filippov, 2020). By focusing on several bacterial receptors, Manohar et al. and Torres-Barceló et al. showed that phage-antibiotic mixtures may limit bacterial growth and antibiotic resistance. They provided an explanation for this by pointing out that diverse

invasion pathways make it more difficult for bacteria to build resistance. Additionally, it has been shown that antibiotics stimulate the development of phage, which amplifies the effects of PAS. Usiak-Szelachowska et al. described the effectiveness of combination of phage-antibiotic treatment against biofilm in 2020 by pointing out that PAS speeds up the breakdown of the biofilm matrix by phage enzymes, which makes it easier for antibiotics to penetrate (Diallo & Dublanchet, 2022). This is a strategy intended to boost the effectiveness of conventional antibiotics by making MDR pathogens more susceptible to them. Selected phages may be used in combination therapy with the antibiotic(s) to which they enhance susceptibility (Nikolich & Filippov, 2020).

In addition, an encapsulated lytic phage cocktail including Methicillin - resistant staphylococcus aureus infecting phages has been produced utilizing nanostructured lipid-based carriers to resolve issues with the formulation and stability of phage cocktail treatment. The cure from MRSA in vivo in rat models was improved by this encapsulation technique, which used a transfersome-entrapped phage cocktail that demonstrated high persistence and stability (Matsuzaki, 2005).

4.3.2. Interaction between host immunity and phage therapy

Studies on how phages and immunity interact are crucial for the appropriate application of phage therapy since there is a possibility for immunological reactions. The site of the bacterial infection and the route of the therapeutic phage injection determine the immune reaction to bacteriophages therapy (Cisek & Dąbrowska, 2016). Anti-phage antibodies identified in the serum of diverse species (e.g., human) indicate a high incidence of natural interaction between animals/humans and numerous kinds of phages (Wright & Hawkins, 2009). Some researchers claimed that the immune system causes the phage level in the human body to decrease following multiple administrations of the same phage, while others claim that similar challenges have no negative effects (Broncano-Lavado , 2021). In phage treatment, oral and intravenous delivery of phages cause the development of antibodies, and phagocytic cells quickly remove phages from the blood and internal organs whenever there are no host bacteria for the phages (Cisek & Dąbrowska, 2016). However, some research findings also claim that during phage therapy, phages are capable of inducing specific antibodies (neutralizing antibodies) against them, which typically hinder phage efficacy to inhibit the targeted bacteria in vivo. Phage preparations have been shown in several other studies to stimulate innate immune response, which is beneficial in the elimination of bacterial infection. (Cisek & Dąbrowska, 2016, Wright & Hawkins, 2009) Research has shown that among the most

significant reasons contributing to the effectiveness restriction of phage treatment is likely the presence of anti-phage neutralizing antibodies (Sulakvelidze, 2001).

The dose regimen and the mode of phage administration (topical and oral administration result in a little rise in antibodies) are two factors that affect the level of neutralizing antibodies (Wright & Hawkins, 2009). Due to the fact that the pharmacokinetics of phage activity are significantly quicker than the host's generation of neutralizing antibodies, research findings have shown that the formation of neutralizing antibodies shouldn't pose a major issue during the first treatment of acute infections. However, if anti-phage antibodies are still detectable when the second round of therapy is given, they could be a problem (Wright & Hawkins, 2009). Yet, since phages work so quickly before the immune response has been formed, researchers do not see it as an issue. However, overall findings imply that it is crucial to examine each phage's immune response, especially if intravenous treatment is being proposed (Cisek & Dąbrowska, 2016).

Chapter 5: Control of airborne nosocomial infection

5.1 Airborne Infection in ICU

Patients and healthcare employees who are more vulnerable than others in the intensive care unit (ICU) might get nosocomial infections from these bacteria that are transmitted through the air (Mirhoseini & Nikaeen, 2016). However, a variety of factors including icu ward activity, density of population, ventilation effectiveness, and environmental variables, may have an impact on the quantity of airborne bacteria in hospital surroundings (Saran & Gurjar, 2022). When a patient coughs, sneezes, talks, speaks, or simply breathes, pathogen-filled droplets are released into the air. These droplets afterwards evaporate in the air and generate droplet nuclei, which are the tiny particles this might hang in air. Wei studied the processes by which respiratory droplets are produced and released from mucus into the mouth. These droplet nuclei might easily be breathed into the lungs since their diameters are typically smaller than 5 μm (Qian & Zheng, 2018). There are numerous studies demonstrating the prevalence and spreading of these pathogens in hospital settings, particularly in critical care units (ICU) and burn units, and indoor air quality (IAQ) is a significant determinant in hospital-acquired infection (Fard & Aali, 2019), managing airborne pathogens should therefore be taken into consideration while designing, setting up, and maintaining hospitals and ICUs (Mirhoseini & Nikaeen, 2016).The Centers for Disease Control and Prevention (CDC) recommends two methods for preventing the transmission of infectious droplet nuclei and reducing their accumulation in healthcare facilities: administrative measures to lower the risk of infecting uninfected individuals through contact with infected individuals and the use of technical measures to limit infectious droplet nuclei and decrease their concentration (Adams & Johnson, 2010). However, to establish more efficient preventive tactics against the transmission of airborne hospital-acquired diseases, an adequate ventilation system in accordance with the requirement and state of patients residing in the ICU is required for control and efficient prevention of antibiotic-resistant nosocomial infections (Saran & Gurjar, 2022).

5.1.1 Airborne Transmission Precautions: Ventilation

Precautions against airborne pathogens are aimed at preventing the spread of disorders that are spread by contaminated small particles or droplets nuclei. A dedicated room with distinctive design and ventilation concerns should be provided to all patients who need airborne protection (Bearman, 2006). One of the most crucial methods for preventing the spread of airborne infections is ventilation, which does so by eliminating or diluting pathogen-filled aerosols

inhaled by ill individuals (Fard & Aali, 2019). However, the availability of isolation services, for instance negative and positive isolation, is the main benefit of mechanical ventilation in the ICU from the viewpoint of infection control measures. Principal components of the ventilation system include the air-pressure difference between the patient's room and the unit, air filtering, and airflow. At the absolute least, air in the ICU must be 99% effectively filtered down to particle diameters of 5 µm in the critical care area (O'Connell & Humphreys, 2002, Crawford, 2021). However, the HVAC system plays a important role in ensuring high indoor air quality (IAQ) in an ICU environment by removing pathogen-laden particles from the air to decrease the likelihood of cross-infection and to provide pathogen-free clean air for inhalation (Saran & Gurjar, 2020, Fard & Aali, 2019). In the ICU, operating room, burn unit, and other hospital settings, enhancing air quality is a somewhat successful technique for limiting airborne infections, according to Gao's analysis (Fard & Aali, 2019).

5.1.2 HVAC system in ICU setting

The principal goal of a HVAC system for a hospital or intensive care unit is to make the atmosphere safer for the patients and the personnel (iHFG, 2020). In an ICU setting, a reliable HVAC system delivers its different functions, including cooling, heating, air distribution, air filtration, air flow rate, and air exchange rates, according to the environment, clinical demands, and patient necessities (Saran & Gurjar, 2020). This system is particularly effective in neutralizing and eliminating indoor air pollution, as well as at removing dangerous chemicals and airborne bacteria and microbes (iHFG, 2020). Air handling systems (HVAC) inhibit the transmission of bacteria and allergens after they have entered a building. This is true for both droplet-borne (including pertussis, coronavirus, *influenza* and *Staphylococcus aureus*) and airborne infections (Saran & Gurjar, 2020). Numerous studies have shown that HEPA filters in healthcare institutions and ICUs may reduce the likelihood of aspergillus as well as other infections; thus, by establishing, maintaining, and improving current ventilation systems with hospital environmental and administrative controls, hospital-acquired infections caused by airborne infectious microorganisms can be reduced (Arıkan & Genç, 2021).

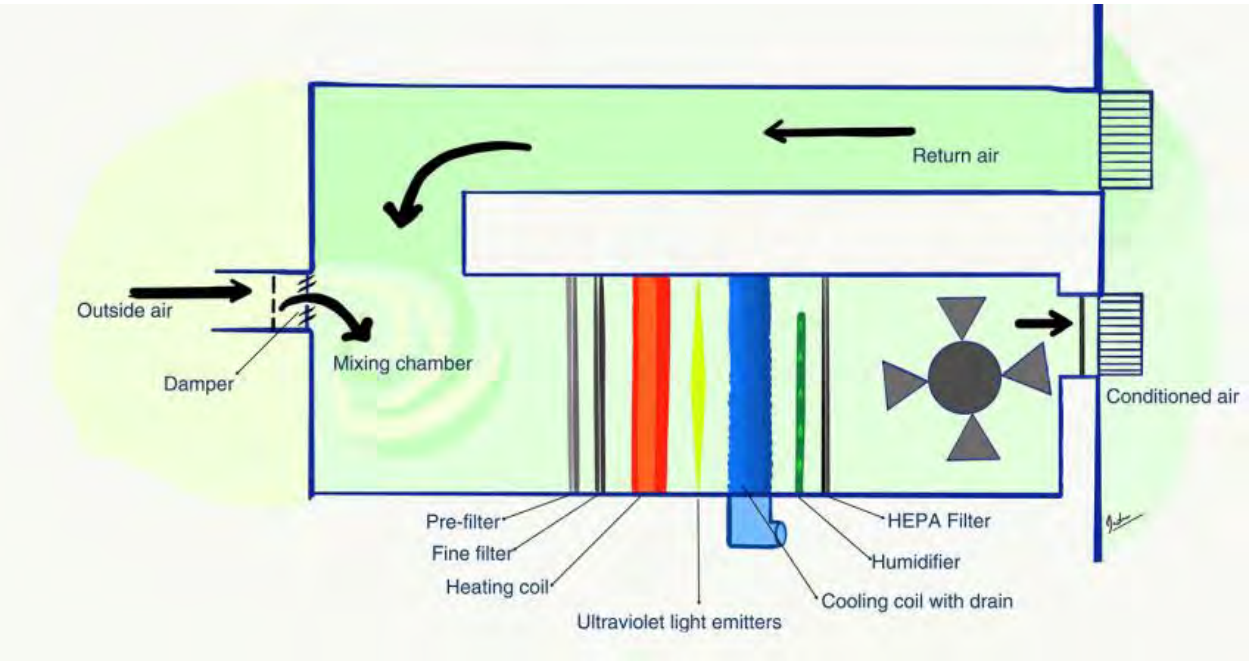

Fig 6: Basic structure of HVAC system.

5.2 Plan of protective intensive care unit

An intensive care unit seeks to manage the airflow in the room such that the quantity of airborne infectious particles is decreased to a level that makes cross-infection improbable (IHFG, 2022). ICUs must isolate patients in order to secure patients from the outside environment (positive pressure) and to limit the transmission of infections from the patient to the surrounding environment (negative pressure) with appropriate auxiliary medical equipment (Saran & Gurjar, 2020). Compared to other patient spaces, critical care units feature quite high air exchange rates and The temperature and relative humidity in the room are also managed (Saran & Gurjar, 2022). It is advised that all air in critical care units with enclosed patient modules should be screened to 99% effectiveness down to 5 microns, and that each module's temperature be capable of being adjusted to a range between 16 and 25 degrees Celsius (Anghel et al., 2020). This may be accomplished by regulating the quality and amount of incoming and exhaust air, maintaining differential air pressures between neighboring regions, developing airflow patterns for particular clinical activities, reducing infectious particles with huge air flow rates and air filtering utilizing HEPA filters, etc (Izadyar & Miller, 2022).

There are the following kinds of intensive care unit facilities: Class P rooms which have positive room air pressure, protecting immune-compromised patients from airborne transmission of any disease; Class N rooms, including anterooms, have negative room air pressure, protecting others from airborne transmission from patients who may be an infection

concern (IHFG, 2022). When the entrance to the critical inpatient unit is opened, contaminated air may flow in and out, therefore it is important to have an anteroom to prevent the loss of pressure which act as a barrier to cross contamination (Saran & Gurjar, 2022).

5.2.1 Anterooms

An anteroom is a regulated space where personnel, equipment, and supplies may move around without contaminating the nearby healthcare facilities. When the anteroom entrance is opened, it serves as a safeguard against the possible loss of pressurization and regulates the entrance or escape of contaminated air (IHFG, 2022). When an immunocompromised patient has to be isolated from airborne infections (in a safe environment), an anteroom is necessary (Andalib et al., 2022). Before entering or leaving the isolated area from the anteroom, personal protection equipment (PPE) or clothes may be put on or taken off (IHFG, 2022). There must be a 2.5 Pa pressure difference and 6 air changes per hour between the patient room and the anteroom. In a demonstration research that examined an ICU's effectiveness in a fully functional healthcare environment, the introduction of an anteroom in the setting decreased the net movement of particles from the critical inpatient unit into the hallway. In contrast to the United Kingdom and certain Australian states, the United States does not need anterooms between critical care units and neighboring hallways for negative pressure isolation rooms (Andalib et al., 2022).

5.2.2 Class P- Positive pressure room

Class P is appropriate in all protective settings with critically sick and immunocompromised patients to prevent the spread of airborne pathogens (Saran & Gurjar, 2020). Since they are kept at a higher pressure than the surrounding environment, Class P - positive pressure isolation rooms need air to circulate from the "cleaner" region into the adjacent space (through doors or other entrances) (Saran & Gurjar, 2020). In a similar manner, the adjacent space is positively pressurized in proportion to the corridor; as a result, air is pulled into the corridor when the door from the adjacent area is opened (IHFG, 2022). According to regulations, air pressure should be kept positive in proportion to any adjacent rooms by delivering 10 to 15 percent more air than necessary and keeping positive room air pressure more than 2.5 Pa in relation to the hallway, but ideal pressure is 8 Pa. (Saran & Gurjar, 2020). The HVAC system does this by introducing more air into the "cleaner" area than is being actively extracted from the same space (Saran & Gurjar, 2020). According to USA recommendations, the room must have enough ventilation in order to achieve a relative humidity level of at least 35% and a ventilation rate of at least 20 ACH. Based on the space's heating and cooling preferences, the ventilation

rate should be set to produce as much air as possible which contributes to the room's ability to have a more uniform air quality by diluting air particles. Saran & Gurjar (Streifel, 1999, Saran & Gurjar, 2020). In order to guarantee particle movement and blending, supply air diffusers should be installed in the ceiling and positioned to send air down into space to a sufficient distance. This clean-to-dirty airflow produces air circulation that should protect the patient from inhaling typical airborne pathogens. Saran & Gurjar (Streifel, 1999, Saran & Gurjar, 2020).

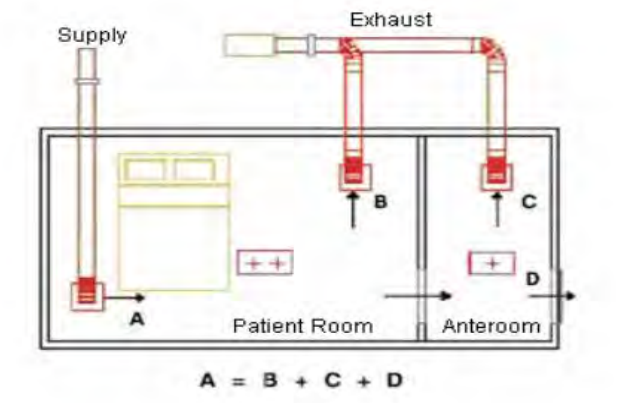

Fig 7: Positive pressure room; in the diagram above, there is an airlock or anteroom next to the patient area. In a room with positive pressure, air would travel from the isolation room to the anteroom and finally to the hallway. By adjusting the major supply and exhaust dampers in response to a signal from a pressure sensor within the positive pressure room, pressure control is regulated (Saran & Gurjar, 2022).

5.2.3 Class N- Negative pressure room

All critical care rooms that accommodate patients who are known to have infections or who are suspected of having infections fall under the Class N category. The goal of this design is to prevent the airborne transmission of infectious pollutants and pathogens into the surrounding environment through an airborne route (Saran & Gurjar, 2022). Negative pressure rooms should be positioned near the entrance to an inpatient unit, so that patients needing isolation do not have to travel through other patient areas in order to reach the critical care room (IHFG, 2022). In an accessible Class N room, for instance, air should travel from the corridors into the isolation room to stop the transmission of airborne contaminants (Saran & Gurjar, 2022). According to U.S. standards, negative air pressure must be maintained between adjacent rooms by expelling minimum 15% more airflow than the supplied system through a separate exhaust system and keeping the air pressure in the room negative with regard to the hallway at or below 2.5Pa (Saran & Gurjar, 2020). The standard air filtration for the negative pressurized cabin is HEPA with UV irradiation on the outflow side and MERV 14 rating air filters on the air intake

side (IHFG, 2022). The room must be appropriately ventilated to ensure 12 ACH. Within the patient room, the air flow should be pulled from a ceiling diffuser positioned near the entrance, while the exhaust air should be taken from lower levels nearly 6 inches above the floor (Saran & Gurjar, 2020). To avoid the possibility of contamination from back draught, exhaust air ducts should be separate from the building's main common exhaust air system (Streifel, 1999). It is suggested that the controls for the isolation room should be visible and reachable by employees so that they may adjust the negative pressure system as needed (IHFG, 2022).

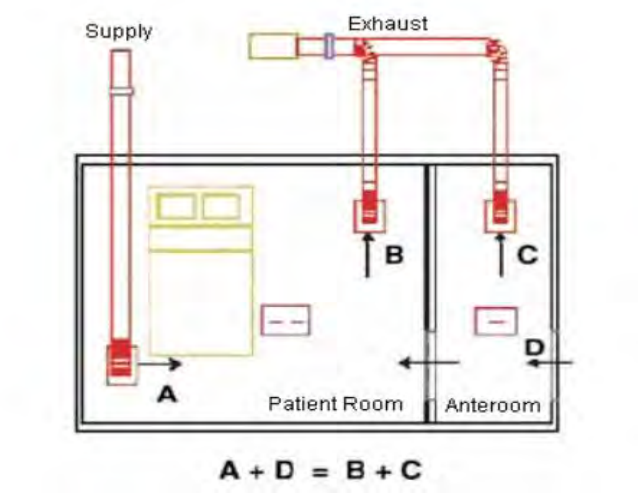

Fig 8: Negative pressure room, The HVAC air flow configuration for class N rooms is shown in the diagram above. An anteroom with the goal of creating an "air-lock" between the hallway and the infected patient. From the anteroom to the negative pressure room, air would circulate. An internal pressure sensor in the negative pressure room sends a signal that is used to modulate the main supply and exhaust dampers, maintaining pressure control (Saran & Gurjar, 2022).

5.2.4 Recommended measurements for positive and negative pressure room

Most hospital systems design and maintain themselves in accordance with the recommendati ons of the Centers for Disease Control and Prevention (CDC), American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE), American Institute of Architects (AIA) and Healthcare Infection Control Practices Advisory Committee (HICPAC) are being followed in the construction and maintenance of the majority of healthcare systems (Saran & Gurjar, 2020).

Table 5: Some recommended measurements for positive and negative pressure room are shown below:

	Positive pressure system	**Negative pressure system**
Direction of airflow	Outside the room	Inside the room
Air change per hour	>20	≥ 12
Pressure	> 2.5 Pa (preferably)+ 8 Pa (ideal)	Less than 2.5 Pa
Supply air	> 20	≥ 12
Temperature	21–24 °C	21–24 °C
Relative humidity	30–60%	30–60%
100% intake of fresh air	No	Yes
Filtration efficiency	Supply: HEPA filter (99.97% 0.3µm DOP)	Supply side: MERV 14 rating air filters (90% dust spot test filters) Exhaust side: HEPA filter (99.97% 0.3µm DOP) with UV irradiation

5.3 Maintaining the air quality standard

Regular air sampling in hospitals, and particularly in ICUs, is necessary to assess the quality of the air inside the room, the effectiveness of airborne particle monitoring, and the efficiency of the air handling system (Anghel et al., 2020). Particle counters that provide real-time air quality monitoring may be used to conduct regular (typically once every six-month) checks of indoor air quality (Streifel, 1999). The most important equipment for detecting particle sizes are those that measure particle size diameters >0.5, 1.0, and 5.0 pm per cubic foot. This

information is particularly helpful for confirming the efficiency of filtration or infiltration in a sensitive environment prior to occupants' arrival. Identifying the cleanest places may be done with the help of these equipment (Streifel, 1999). Additionally, air is evaluated for its moisture levels and its relationship to air temperature in order to keep it within the approved range (Fard & Aali, 2019). The ICU belongs into the "extremely high-risk category," where vital maintenance criteria must be met. In order to do this, in addition to educating the ICU working crew on the facility, it is essential that the engineer construct these parts to be readily accessible. For the HVAC system to operate properly, each component, such as outdoor air (OA), return air (RA), supply air (SA) and exhaust air (EA), as well as the pressure difference, particle count, temperature, and relative humidity, should be clearly labeled (Saran & Gurjar, 2020).

Chapter 6: Discussion

The development of novel, highly effective treatment options to treat bacterial infections caused by resistance to antibiotics in critically ill intensive care unit (ICU) patients is urgently needed due to the rising worldwide issue of antibiotic resistance. Antimicrobial resistance is currently on the upswing due to widespread and excessive antibiotic usage by patients. The emergence of bacteria that are tolerant to antimicrobial medicines is currently the most challenging issue in the treatment of bacteria-related disorders, consequently awareness is being turned to a different potential therapeutic, such as bacteriophage therapy. Similar to antibiotics, bacteriophage therapy is not a miracle cure and should not be administered in the spot of antibiotic therapy. Rather, it should be thought of as an adjunctive therapy that may be used to cure disease that are resistant to antibiotics as well as non-urgent situations where the administration of antibiotics should be overlooked to prevent the incidence of resistance.

This systematic review presents information on efficacy and safety, as well as a few noteworthy results from human Bacteriophage treatment research that have been documented in papers published over the last 10 years. According to the results, it can be said that Bacteriophage therapy achieved positive outcomes for the management of infections brought on by a variety of bacterial species, particularly those that are challenging to manage, including disease caused by bacterial resistance to numerous antibiotics. Moreover, Lysin is also a suitable substitute among antibacterial treatment alternatives through encapsulation, and it may also be implemented to develop novel phage-based medications. Regardless of whether it is slightly expensive, it may be a vital step for the initiation of phase treatment establishment. However, there are still some limitations to bacteriophage treatment. For instance, few have known about how successful this therapy is, and the stability and efficacy of its formulation are still in doubt and sometimes don't provide the optimum results. Additionally, one of the major factors with PT is phage resistance, although it may be controlled by incorporating various phages in cocktails or combining phage and antibiotic to expand the phage's host range and increase the number of targets that might potentially increase the effectiveness of therapy. Immunological responses are still another issue, but researchers do not see them as a concern since they may be resolved by changing certain parameters. Phage treatment for infections is now being studied, along with ways to succeed in making it more stable, effective, and popular in the years ahead. Phage treatment therefore is not authorized in the US and many other countries yet. Our hope is that more studies and clinical trials on this subject are ideally necessary in order to make the treatment more available to the public.

On the contrary, most nosocomial infections were previously believed to be transmitted by direct contact; nevertheless, researchers have discovered compelling evidence indicating airborne transmission plays a predominant role in developing nosocomial infection. Poor air quality is the cause of 50 percent of all life-threatening nosocomial infections and pneumonias occurring in critical care units (ICUs), despite ICUs accounting for just 15 to 20 percent of all hospital beds. For the control and prevention of life-threatening healthcare-associated infections, the construction of effective HVAC systems that manage the quantity and quality of exhaust and intake air, create airflow patterns for specific clinical operations, maintain different air pressures between adjacent areas, dilute infectious particles with massive air supply, and filter air using HEPA filters should be emphasized. ICUs must isolate patients in order to keep them safe from the outside world and to stop the transfer of pathogens from the patient to the outside environment. Positive pressure rooms and negative pressure rooms may be implemented in ICUs to accomplish these goals. The critical care unit does not provide the proper environment for seriously sick patients, which results in an elevated death rate, according to various studies that have indicated that hospital ICU structures in developing countries do not entirely meet the authorized requirements. In the United States, pressurized critical care rooms are only available in 2% to 4% of all hospital rooms. By maintaining an optimal range for the concentration of airborne particles, positive and negative pressure critical care rooms maintain air quality that is suitable for both patients and medical personnel. Nevertheless, it is crucial to keep infections under control in all hospital settings, hence every country's regulatory agency must be strict regarding the implementation of well ventilation systems in ICUs with adequate pressurized rooms for preventing airborne nosocomial infections.

Chapter 7: Conclusion

With the emergence of multi-drug resistant nosocomial infection, eliminating infections at their source or identifying an alternate form of treatment is the most reliable approach to reduce the probability of rising morbidity and death rate caused by serious diseases that are now incurable. Here, prevention and treatment are crucial points to concentrate on in order to solve this issue. Adhering strictly to infection control procedures and installing recommended pressurized critical care rooms to reduce cross contamination are the keys to controlling airborne antibiotic-resistant infections in the ICU. Bacteriophage therapy, on the contrary hand, is a potential and safer approach to manage multi-drug resistant nosocomial infections. But current knowledge is inadequate to permit the usage of this therapy. To clarify the stability, safety, and effectiveness of bacteriophages therapy for treatment of bacterial infections globally and remove some of the drawbacks we have outlined, more study is required to determine the exact formulation and roles of phages therapy in disease treatment.

7.2 Limitation of the study

This study has limitations due to-

I. complete dependence on previously published research, some of which are not current.
II. limited access to some documents due to publisher restrictions.

7.3 Future research plan

Bacteriophage therapy has a promising future. The future of phage therapy relies in the hands of scientists to succeed in making it more stable, effective, and popular in the years ahead. Phage treatment has the support of the FDA. The National Institutes of Health (NIH) of the United States has provided funding to 12 institutions worldwide to research on phage treatment and develop it as an alternative of antibiotic. Well-planned clinical trials should be designed to analyze the effectiveness and safety of bacteriophage therapy. On the contrary, with the constant development of technology, there is a need for updated infection control standards and guidelines that include uniform criteria for well-ventilated, pressurized intensive care units.

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